UNRESTING CELLS

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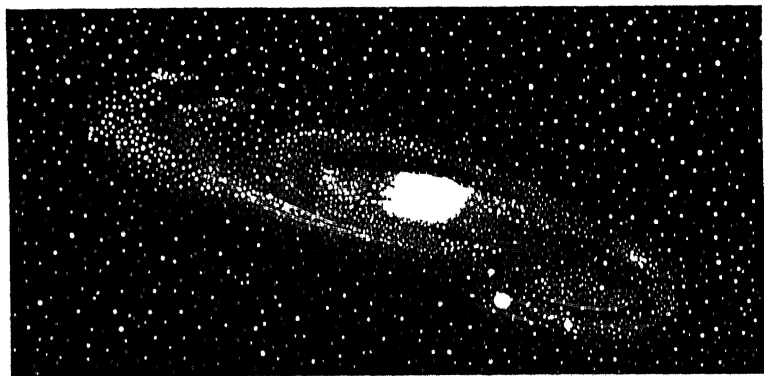
Dedication

Each generation receives from the past, slightly modifies its heritage, and passes it on to the future. This volume stems from great teachers who have molded my scientific individuality. I would dedicate it particularly to:

MAURICE GERARD, who showed me early in childhood the power and beauty of a rational approach to phenomena;

JULIUS STIEGLITZ, who opened for me the vistas of chemistry;

ANTON J. CARLSON, whose healthy skepticism was a guide into the difficulties of physiology;

A. V. HILL, who taught me something of the quantitative and analytic approach of physics;

RALPH LILLIE, whose broad and deep insight into the meaning of living organisms and life processes has especially tinctured my own thoughts on these matters.

"What am I, Life? a thing of watery salt,
Held in cohesion by unresting cells . . . ?"

John Masefield

Contents

Foreword

The most valuable thing in the world is life, and the most valuable life is that of man. If we accept these propositions, what is more important in gaining an appreciation of our place in the world than trying to understand what life is? It is this formidable and entrancing problem which is the theme of *Unresting Cells*.

Professor Gerard has brought to bear upon this question a knowledge and familiarity with the subject gained by years of active research. His facility as a teacher shows itself in the richness of simile and illustration which clarifies his presentation. He has traced the life of the cell from its primitive beginnings, through the complex chemical processes and intricate physical mechanisms, to its functioning as a part of a complete organism. Man himself is in a sense the culmination of this development, but he in turn is a unit in the yet greater organism of human society. With rare perspective and appreciation of important detail the author thus presents a fascinating picture of the processes of life.

What is it that distinguishes living from non-living matter? How does the enzyme perform its intricate and wonderfully efficient tasks? What are the chemical changes that occur as a cell absorbs its food and grows? It will be difficult for the non-specialized reader to find elsewhere so clear an account of these remarkable phenomena.

There will be those who ask, how in these difficult days when civilization itself hangs in the balance can men be con-

cerned with details seemingly so remote from the task before us. E. H. Lewis had heard this question when he wrote:

> See on the wall the wind-swayed ivy leaf;
> Will brave men pause to name its useless parts,
> And listen not to what the wind doth bear—

> The sobs of human grief,
> The noise of battle and the knell of hearts,
> And all the frustrate world's unanswered prayer?

Is not our increasing knowledge that which distinguishes man from beast? And is it not in a world whose thought is free that we have thus found our chance to grow?

With the world at war, our nation bears a double portion of the burden of maintaining and advancing that knowledge which is man's most precious heritage. Professor Gerard has clearly seen that only as the truths of science are widely shared can their value be appreciated. Correctly he sees as the task of science not only to give man the strength that comes with specialized skill, but also the understanding that makes him human. He has given life to the story of life and for his readers has thus made life the richer.

                                    ARTHUR H. COMPTON

Chicago, May, 1940.

Preface

Biology occupies a middle place in knowledge. Based in part on physics and chemistry, it would attain a lucid analysis of living things similar to that being achieved for the incomparably simpler systems of the inanimate world. Yet, like its more ambitious sisters dealing with the even subtler complexities and manifestations of the human mind, it has great tracts in its domain awaiting discovery. A rigid formulation of relationships can hardly be achieved when the phenomena to be related are scarcely known; and many adventurous explorations and major discoveries remain to be made. But while the frontier of our map is extending, older regions are being intensively developed. At the same time that the complex and amazing beauty of organisms is unfolding, a no less exhilarating understanding of the underlying processes and mechanisms of their being has come into existence.

It is not so difficult to describe the form and behavior of creatures in a way to capture the interest and intrigue the imagination of an intelligent person, and many excellent presentations of biology from this angle are available. More difficult, and rarely attempted, is an analysis of how they work, directed at the understanding. This is a formidable task, indeed; for the analysis soon leads to the microscopic cell, submicroscopic particles and films, complicated molecules and forces—phenomena far beyond the comfortable familiarity we all have with cats and caterpillars and crayfish, or with even hearts and brains and muscles, though we may never have seen them. Yet

the uninitiate have been whisked through the far end of a
telescope and sent prowling through interstellar space and
light-years to the farthest regions of the universe and returned
ungiddy and enriched from their celestial journey. An equally
rich adventure through the microscope is possible; one as sur-
prising as Alice enjoyed when she went through the looking-
glass.

Some nine years ago, when asked to contribute a guest edi-
torial to an urban newspaper, I chose to discuss the problem of
popularizing science and wrote:

"The story has it that, after Scheherazade had entertained
the caliph with a thousand tales of genii and talismans, she
described a strange and wondrous beast for him—a modern
ocean liner. The caliph found his credence so painfully
stretched that only the execution of his charming raconteuse
could restore his tranquillity.

"We are in these days more sophisticate; not to the degree
that marvels no longer titillate our imaginations, but in that
magic lamps have passed beyond the boundary of our credence
and ether waves have entered it. So science supplies the
Arabian Nights of the twentieth century.

"Nor would I quarrel with the vicarious thrill which Mr., or
more often Miss, Everyman manifests when rhapsodizing over
the latest 'conquest of science.' 'Isn't it marvelous, this door
which opens on command?—a modern sesame!' Such enjoy-
ment is a positive gain to the enjoyer and, of itself, harmless
enough to all.

"The danger is, of course, that a few clever tricks or applica-
tions come to be for millions a symbol of science. Einstein and
Houdini become fused into a picture of the scientist-miracle
man. The reader of such science news items is only concerned,
or at least the writer of them assumes he is concerned, with
enjoying a gentle tingling of his imagination, a sort of mental
Turkish bath. Active exercise of his intelligence, to appreciate

the significance of or principles behind a real scientific development, is neither expected nor aided.

"Further, 'science' has at last become respectable enough to take its place in parlor conversation; and current news agencies, dailies and weeklies especially, purvey such bits of information as come to hand as new and fruity morsels for their patrons to mouth. It matters not at all if they be stale for years, or wildly unsound, or merely infinitely trivial. The item is the thing and, like a precious stone, needs no setting to manifest its worth.

"But science is a mental structure, I might say machine, which turns out factual ingots, occasionally exciting or useful. The facts, gleaned from endless observation and experiment, are embedded in a matrix of relations and reasonings. They are of scientific value as they solidify a theoretical position or form a base for further analysis. Some may yield to utilitarian exploitation—all to the good—but such a value is foreign to those of science (often called pure science). As well judge the brilliance of a game of chess by whether it is played with wood or ivory pieces.

"Anyone with moderate intelligence and interest could enjoy observing the mental agility of the investigator as much as the dexterity of the golfer. There is a thrill for audience as well as performer when a clever stroke drives the ball for many rods to the green, or the insight for many digits to the electron or galaxy. Whether a useful reward lies at the end is a secondary matter. Such a popularization of science is possible but all too rare. We usually invite the layman only to examine the golf balls, or at most the loving cups; why not permit him to watch the game?

"All men know Thomas Edison, the wizard of Menlo Park, though his scientific contributions were minor. They should rather scrape an acquaintance with Michael Faraday, whose brilliant experiments ushered in electrodynamics. He even envisaged the utility of his discoveries—the motor and dynamo

are two applications—though he was not willing to sacrifice other interests to their development; for he is reported to have disgustedly said to Gladstone, who was unimpressed on seeing the experiments, 'Oh well, some day you will be able to tax them.' "

The present volume is a response to my own challenge. It has grown during the intervening time, by fits and starts, in the scant leisure permitted by a full research and teaching program. During this interval, I have become ever more convinced by the sweep of world events, that the scientist—yes, the pure scientist—is not merely justified in expending some energy on the popularization of sound science but, even more, has some duty to civilization to do so. The technological applications of scientific discoveries have unleased a tremendous power upon society. If this power is scientifically utilized—under the same conditions of intellectual honesty, rigid respect for established facts, careful and impartial testing of theories, and drawing of limited conclusions only as facts justify them, that have built the cathedral of science itself—humanity can confidently look forward to new heights of comfort and creation. But if this power is misused by individuals or groups unschooled in the rigid intellectual virtues which are the scientific attitude, humanity may well face catastrophe. Scientists must help recruit men in other walks of life to the use of the method and attitude of science in dealing with problems of state and society.

The intent throughout this volume has been to present the problems that biologists have recognized, the evidence they have accumulated and organized in attempting to solve these problems, the answers which they can give today, and particularly the careful manner in which they reach and check their conclusions. Facts are included which support or illuminate these conclusions, a very few for the sake of imparting interesting information. I have tried to be accurate in factual statements (though undoubtedly errors have slipped in), but with

one proviso. When a first approximation to the truth, as known, could be presented simply and clearly, I have not hesitated to omit those further qualifications and exceptions which contribute to a second and closer approximation but which do not affect the import of the original unqualified assertions. May my fellow biologists, who know that a human red blood corpuscle is not a flat disc (as I have stated), but a biconcave one, and who properly doubt that an amoeba is "hungry" and "searches" for food, be as tolerant as they are able of such simplifications.

The book is not intended as a complete essay on biology but is distorted in emphasis in accord with my own training and interests. The grand sweep of evolution finds scant mention and the physico-chemical aspects of life phenomena are relatively overemphasized. This is deliberate. Animals and especially vertebrates have supplied a disproportionate amount of the illustrative material, on the other hand, largely because I am better acquainted with them.

Many of my colleagues have contributed to these pages, for I have picked their minds over years of fruitful association—in courses in physiology and general biology and in informal conversation. Several scientific friends have kindly read and criticized one or more of the chapters that follow, and I wish especially to thank for their good advice: William Bloom, Ralph Buchsbaum, Paul Cannon, Anton Carlson, Merle Coulter, Alfred Emerson, Edwin Fetcher, Ralph Lillie, Arno Luckhardt, Joseph Schwab, and Sewall Wright. Since, however, I have not in all cases followed their suggestions, the shortcomings of these pages rest solely with me. My thanks are also due a sequence of loyal secretaries, who have helped midwife this creation: Mabel Blake, now Dr. Cohen; Helen Blake, now Dr. Carlson; and Mrs. Esther Scheinman. Mrs. Elizabeth Newhall has helped plan and has strikingly executed the many original illustrations.

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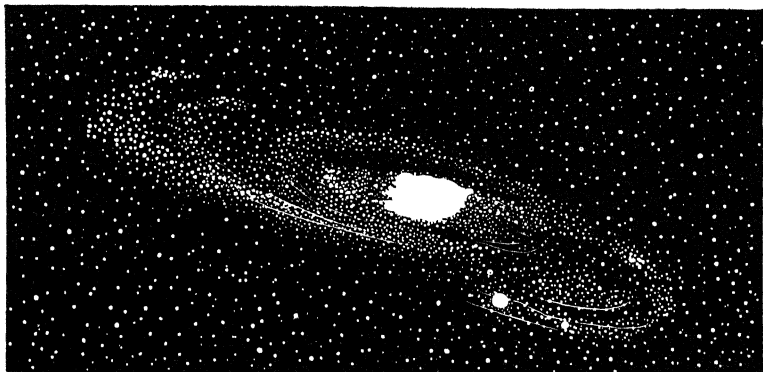
Chapter One: Out of the Nowhere—Prologue

Some time life began. A few scientists have speculated that this beginning was not on this earth, but occurred long before our planet existed and on far distant, probably now vanished, heavenly bodies. Some hypothetical "biospore," having escaped from such a body, and remaining completely dormant and inactive at the zero of all temperature, might have floated for eons through the celestial void until it reached a habitable world, warmed up, started activity, and so became the progenitor of a world of life. Such a voyage might have been made again and again by descendants and ultimately one happening on to earth might have become the Eve of all earthly creatures.

Space, Motion and Life

Of course, the distance from some habitable speck of the universe, a planet of a far sun, to another, our own mite, is enormous. Light itself, the ultimate in speed, measures the distance to our nearest neighbor in years of travel, to the outlying inhabitants of our "island universe" in millennia. A bit of life stuff, probably submicroscopic, once it wafted beyond the immediate pull of its home planet, would float on at the speed and in the direction with which it started, possibly pushed faster

by the pressure of light coming behind it, possibly deviated by chance contact with other lone wanderers in space, until it was swept up by the gravitational force of its home-to-be. An unbelievably long time must elapse during the journey. The span of life of any individual, or species; yes, the whole duration of life on the earth would be a clock-tick by comparison. Ordinarily the precious life stuff would have died en route and arrived inanimate as star dust.

But space is cold. Cold below where water freezes, below the temperature at which mercurial quicksilver becomes set and rigid and can be used as a hammer to pulverize blocks of solid alcohol. Colder than the points where air itself settles and liquefies and becomes hard, than the far greater cold that causes helium gas to do the same. Cold to the zero of temperature where no heat exists.

Heat is necessary to life in an interesting way that must be
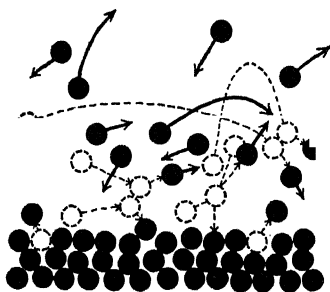

Fig. 1. Molecules continuously dash about, collide, and rebound. Those in a "rare" gas above a liquid (shown below) or a solid—as in the outer atmosphere—move in curves, just as does a thrown ball. Only when one chances to be moving away from the horde of attracting molecules rapidly enough to overcome their pull, does it "escape."

considered and, strangely, to the very question of how the life dust could leave its planet. Heat is really the dance of those minute units of matter, the molecules, as they restlessly jostle and rebound from one another. The faster they go the warmer is the object they compose, not unlike a dance hall with its swirling couples. And, also, the harder they collide, the farther they rebound until, for every substance, some temperature is reached at which the attraction of each particle for all others (gravity) can no longer hold against their restlessness. The family disbands, the community disperses, the substance vapor-

izes, becomes a gas, and its member-molecules fly off from one another into space until stopped by impinging on an obstacle. If huge enough numbers of molecules are close together even as a gas, the pull of the mass gravity is likely to turn back slowly the wayward ones rushing out to empty space, so that nebulae, such clouds of separate molecules, can exist in the skies.

Even more, a large solid body like the earth, with molecules packed several thousand times as closely as in air, can hold about it by gravity a thick blanket of gas molecules, the atmosphere. A single one, somewhere in the swarm, even if it should bounce off another and start directly away from the earth, has no chance to leave, for almost immediately it will collide with one above and, like a frisky puppy, set off with undiminished energy in a new direction. But near the top of the atmosphere, if one should start up, its fate will depend on how fast it is going. The molecule acts like a bullet fired upward which gradually slows, stops, and then falls back with increasing speed. The pull of all the molecules of the earth is steadily drawing upon it. But this pull fades rapidly as the objects separate and, if the small one gets a sufficient start, the pull that slows it fades out before it has slowed to a stop. Then it keeps going into space. For a molecule to escape from our earth, it must start straight away faster than seven miles a second. This may seem a good deal—a bullet leaves the gun at less than a twentieth of this speed—but even at ordinary temperatures many molecules in the air are moving this rapidly, and as it is heated their number increases sharply. However, to balance the air thus regularly if slowly escaping entirely away from the earth, other molecules wandering through space are caught by the long gravitational tentacles and swept into the atmosphere.

What keeps the molecules so lively? That story must be told elsewhere—how radiant energy is caught by them and in turn radiated away, though in truth we know less of how this works

than of how certain radiating strains of music reach slowly pirouetting couples and start them ricocheting about the dance floor. But at least it is true that energy is slowly radiated and, if not made good, the molecules lag on their wayward course. And as the component molecules lag, the composed substance cools. A hot stone in the most perfect vacuum thermos bottle will slowly cool to the surrounding temperature. The whole earth, and each heavenly body, cools in its surrounding vacuum of space (except as the heat lost is balanced by receipts), and from the interior of the globe to the edge of the atmosphere the temperature falls lower and lower. Out in the open oceans of space between worlds the temperature is essentially the absolute zero; there is no heat; the last feeble molecular shivers have ceased; matter exists in a state of ultimate cold and quiet.

Quiet and cold and dead. For life is change, action, movement. The very words and structure of language, even our colloquial slang, recognize this. Animals are "animate," life very much "alive," an excited person is "heated" up, an unresponsive one a "cold" proposition. The agile spring of the cat, the buzzing bee, the bursting willow buds, the gentle breathing of a sleeping child, all are events. Even the microscopic yeast organism sturdily goes about its business of budding new generations and performing its simple magic of sugar-fermentation. Our own muscle movements depend in part on fermentation, occurring in the living muscle, in part on a type of breathing in it. Chemical change is needed to build more of the living stuff in the growth of all animate beings. In living bodies as in nonliving, the molecules dance, collide, react, and only as different kinds of particles, molecules of separate species of matter, come together can they change one another. Breathing, growth, movement result from molecular interactions following these chance encounters of different sorts of molecules.

Life, then, requires chemical change which, in turn, depends on molecular movement, which varies with temperature. Small wonder that wood burns only when hot, that eggs harden in

boiling water and meat keeps in the refrigerator but spoils in the room, for all these are chemical changes. And one can tell quite exactly the temperature in the open by counting the number of chirps a cricket makes in a minute or how many bees leave the hive or the rate at which the heart of a small fish beats or by measuring how much carbonic acid is formed by a cake of yeast in sugar water; for the speed of life, like all chemical interactions, is under the control of temperature. How excessive heat kills, we must consider later; but cold has one clear enough effect. As its leaden fingers subdue the active particles and the rhythm of the dance slows and fails, change is suspended, life hangs still, and time itself pauses in its eternal unwinding.

The tiny biospore, somehow jostled away from its birthplace —where, of course, conditions were such that life could and did flourish, the particular wanderer being but one descendant of a race of living things stretching still farther into the cave of antiquity—the biospore, almost at once on entering the heatless void, would itself cool down to absolute zero, and so to changelessness and death. Utterly quiescent, it could not age or molder. Its own clock stopped, it is immune to time; though relatively to other objects in the universe—the mutual approach with the planet it will ultimately meet—the hourglass still runs. Then the frozen mite may drift over parsecs and for eons, with time or change passing by but not through it, and so arrive at long last on the earth just as it started on the journey, as if conveyed by a supernal refrigerator freight.

But well-frozen bulbs will never give forth blossoms nor frozen fish glide through the water when they are again made warm. They are dead irrevocably, irreversibly. Death is no
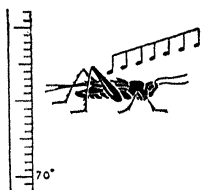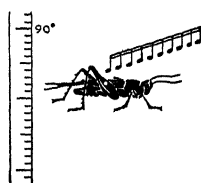

Fig. 2. One can tell the temperature outdoors by counting the rate at which a cricket chirps.

simple alteration, and many bodily changes are induced in the more complex organisms by freezing. Even in the simplest ones, a sudden freeze to temperatures sufficiently below 32° Fahrenheit (or 0° Centigrade) will cause the water, present as it is in all living material, to crystallize as ice. As it solidifies it swells and ruptures any container that opposes it—the steel shell of an automobile radiator, the glass wall of a milk bottle, the delicate veils and membranes that enmesh all living stuff. Thawing will not undo this damage, and most animate beings if cooled only enough to freeze their water, five to ten degrees below the Centigrade zero, are beyond restoration. But not all. (Even fish, if frozen very suddenly so that the ice crystals are extremely small and do not poke through cell membranes, may recover on warming.)

The bacteria that cause typhoid fever, stubby rodlike bodies
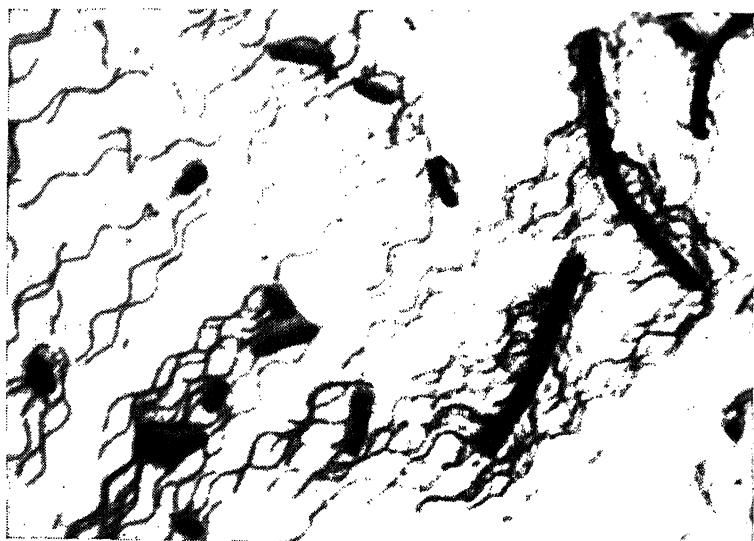
Fig. 3. Microphotograph of individual typhoid bacilli. Enlarged several thousand times. The bacteria are treated to show the whip-like threads they use for swimming about.

1/8000 of an inch long, are one in-
teresting exception. They have
been especially studied, for these
organisms pass with sewage along
water courses and, unless killed,
may cause wide epidemics. Appar-
ently they can survive a winter in
solid ice high in the Alps, but it is
not easy to be certain how cold
they really become there. [Simpler
to do the test in a laboratory.] Bac-
teria obtained originally from ty-
phoid patients have been kept alive
and growing by placing them on
sterile gelatine containing proper

FIG. 4. Typhoid bacilli,
touched to the surface of a
jelly by a sweeping stroke of
a wire carrying them, grow
into colonies with great num-
bers of individuals in each.

sugars, meat juice, and the like. After some days a bacterial
mass or colony forms. The tip of a wire touched to this and
then on to some fresh jelly carries enough individuals to col-
onize the new country (the jelly surface is to one of them
as large as Switzerland to us), which they promptly do; and
the transfer may be carried on indefinitely. And if ultimately a
transfer is made back to a healthy man, which is not an infre-
quent accident in bacteriological laboratories, the germs are
likely to grow in him as in the jelly. The chemical and other
changes produced by the typhoid bacilli, plus the activities of
the man's body in attempting to counteract these, constitute
together the disease, typhoid fever. It is easy, then, to test for
living typhoid germs. If they grow and form colonies on proper
jellies or other media for growing or culturing them, and if they
produce typhoid fever when administered to rabbits (which can
be given a disease not unlike that in man), then they must be
alive.

Suppose now that several portions of the same culture
medium are inoculated at the same time from a well-grown
colony of these bacteria. One is then left on the table, one

placed in the refrigerator, and one in an incubator at 37 Centigrade, the body temperature of man. At intervals each is examined. The little, clear, dewlike drops on the jelly's surface that constitute each a nation of typhoid bacilli appear in the warmed portion in a day, in the untouched one after weeks, in the one kept cold not at all. Clear enough: the speed of life varies with temperature, and growth is but one life activity slowed by the cold. Then the cold portion should grow well enough when put, even months later, in the incubator, and so it does. But what of life and death here? The refrigerator may be cold enough to freeze the bacteria, but still molecular movement continues and the vital activities, though slowed, may trickle on. The cell structures may yield rather than crack before the swelling ice. The bacteria then are dormant, not dead; their lives run feebly, but they run. We must make the test more severe.

Start again with a rapidly growing culture of typhoid germs. Cool it gradually in the cold box to five or ten degrees below zero Centigrade. Put it then on "dry ice"— carbonic acid gas frozen solid— until it cools to 78° below zero, then in liquid air which is far colder, minus 182°. Do not stop, but place this in liquid hydrogen. The temperature falls to minus 252° and even the liquid air turns solid. The cold is great, the experiment becomes ever more diffi-
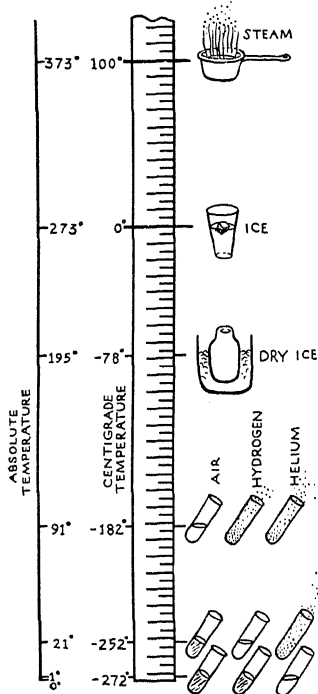

Fig. 5. The lower range of temperature, to absolute zero. There is no top for heat; star temperatures are known over 30,000°C.

cult to pursue, for the ice box is now a furnace compared to
the frozen air, and special insulation is needed to keep it all
from boiling violently away. But still the experiment can be
pressed farther. Liquid helium, even solid helium, has been
obtained at a temperature 272° below zero Centigrade or 460°
below zero on our ordinary thermometer. Into this put the
bacteria to cool down, down to within a degree of the absolute
zero, the temperature of interstellar space. Now surely life is
stopped, completely.

Then begins the slow return from this edge of existence. The
helium is allowed to boil away, the hydrogen, the air, the
carbon dioxide. The water melts, the germs are returned to the
room temperature. Do they live? A number of them are trans-
ferred to a fresh medium and put in the incubator; a rabbit is
infected; we wait. And then they appear—the dewdrop colonies
on the medium, the fever and bloody diarrhoea in the animal.
Truly, by warming up the congealed germs we have made life,
for they were stone dead in the terrible cold. Could not the
biospore after its timeless voyage through space likewise
revive? Why not?

The Rise of Protoplasm

Some minds find surcease from the gnawing uncertainty of
problems which, like that of the origin of life, are beyond cer-
tain solution by relegating them to a sufficient temporal and
spatial remoteness. The yearning for a dim discernment of how
it might have been is assuaged if life began so far away and
long ago as to be almost beyond the vision of the mind's eye.
The problem then seems less acutely imminent and settles com-
fortably into the limbo of accepted axioms. Yet there the chal-
lenge stands: How did life *start*? This is a more searching ques-
tion than where? or when? All the above discussion indicates
only that this planet *might* have been colonized from another,
not that it *was*. In fact, the earth is peculiarly suited in its tem-

perature, moisture, and other properties to support the sort of life we know, and equally to originate it.

Reading back from the present the fossils in the rocks, the traces and fragments left by living things, their pattern becomes always simpler, and the saga breaks and vanishes some half a billion years ago; for the early fragile, naked bits of life substance, devoid of bone or shell, would leave no certain token of their presence able to withstand the mighty forces of destruction and change swirling upon the earth's surface for millennia. Yet, even then, a wide array of living forms already existed; change and divergence by descent, through generation succeeding generation, had been long in action and individuals had deployed to a great range of habitats. There is no reason to doubt a similar continuation backward, the panorama narrowing to ever fewer kinds of simpler beings, and in fact even a billion years earlier there are deposits suggesting the presence of a most primitive kind of life.

The tree of life, then, robust and branching as it is now, took root on earth perhaps halfway back to when the earth itself had a solar birth and from the simplest, most unpretentious start. So must the play open, wherever the setting. Perhaps life appeared *de novo* only once and later spread throughout the heavens. Perhaps it still is limited to our earth. Perhaps it formed and will form again and still again when and wherever the needed stage be set. Perhaps, indeed, it is forming even here and now only to be consumed when hardly started by other established organisms. On earth the active start may have been a biospore wafted from beyond or a similar bit of life-stuff formed at home.

What sort of thing would qualify as life-stuff or protoplasm (first fluid)? Though innumerable protoplasms exist today in the many parts of varied organisms, they all show such an astonishing similarity in characteristics, the protoplasm of a typhoid bacillus so closely resembling that of the human brain, as to enhance the belief that similar properties were present in

the earliest protoplasm at the start. Yet this is no small require-
ment, for both in its physical architecture and in its chemical
composition, protoplasm is superlatively intricate and complex.

To be sure, only the well-known elements are present, mainly
the most common ones: oxygen, composing in air, water, and
rocks half of the earth's surface bulk; hydrogen, held in huge
reserves in the water of oceans; carbon, emerging originally,
perhaps, from volcanic vents and still present in small amounts
in the atmosphere, now spread in huge layers of limestone over
the earth (mainly by the labors of earlier living forms), and
leached from them slowly by the land waters; nitrogen, com-
prising the bulk of air and locked in many rocks as saltpeter
deposits; phosphorus, sulphur, iron, sodium, potassium, cal-
cium, chlorine, iodine, etc., all plentifully present in rocks and
ores and waters, comprising, in fact, in their various combina-
tions with one another the salts of the salty seas.

But what slight comfort is this, that no special elements need
be conjured up for the matrix of life; for the great majority of
substances present in protoplasm are formed by particular com-
binations of these elements, are composed of molecules contain-
ing the simple, familiar atoms of the elements in unique ar-
rangements. Such substances are almost unknown in nature
except as produced by living organisms. Of course, there is
nothing mysterious about these organic substances—by which
name chemists still denote them, a heritage from the time, a
scant century ago, when their synthesis seemed beyond hope
—for they are made in ever-increasing numbers in the labora-
tory. But at what a cost of planning, care, and understanding!

Sugar contains only carbon, hydrogen, and oxygen atoms,
and is the stable currency of the world of life. The meanest
plant builds it from carbonic acid and water, a dilute carbonated
water present everywhere, whenever the sun smiles down its
energy upon the green pigment. The tiny pond slime, like
bacteria a simple microscopic blob of protoplasm but containing
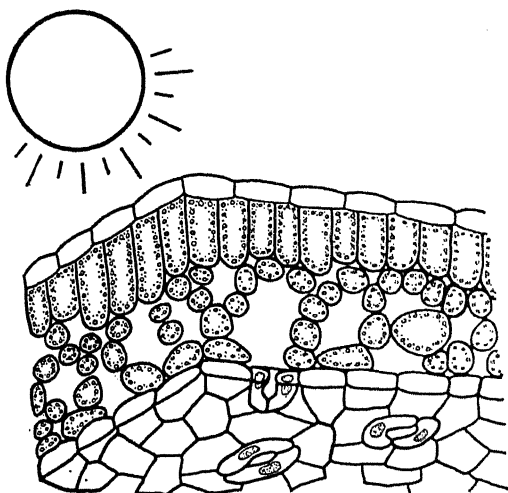the precious "leaf green" or chlorophyll, changes the car-

FIG. 6. Magnified section of a leaf, showing the chlorophyll-containing cells on the sunny side and the "air pores" on the under side.

bonated water without effort to sugar, starch, protoplasm, so long as the sun gives light. It makes it in surplus, stores it, burns much for food, becomes itself food for amoebae or other minute animalcules which also play a skilled game of chemical handball with the sugar molecules. No bit of living protoplasm is so clumsy as to fumble this game; all are adepts at the alchemy.

The chemist also can make sugar. There are many chemical roads to the sweet substance, all the way from these simplest beginnings. But they are devious winding paths, often precarious, advancing by slow stages from one outpost of combination to the next more complicated one, itself in turn the start for a struggle to the next intermediate product. So, laboriously, is atom attached to atom in proper sequence and correct arrangement until the complete sugar molecule has been forged. It can be done—with the full armamentarium of the laboratory conscripted into service, with the masterly strategy gleaned from centuries of study focused on the problem, with willing hands laboring long months. And while the scientist works to his

truly transcendental achievement, culminating in a few small transparent crystals of sugar formed in his last reaction mixture, the ivy leaves overhanging his laboratory window, perhaps casting a wavering shadow on the historic test tube, have made pounds of this same sugar and built it and other far more elaborate stuffs into the protoplasm of ivy leaves, or turned it into wood, or simply burned it up again.

If the letters of the alphabet are likened to the atoms of various elements, then the molecules of compounds are words. Water molecules, made of two hydrogen atoms to one of oxygen, are HOH; vinegar acid (containing carbon as well), CHHHCOOH; alcohol, CHHHCHHOH; ether (the methyl variety), CHHHOCHHH. Alcohol and ether, note, have the same number of C, H, and O atoms, but are as distinctly different from each other as are "each" and "ache" with the same letters. The simplest ordinary sugar has six carbon, twelve hydrogen and six oxygen atoms in each molecule, a twenty-four lettered word. Common cane sugar has two of these words combined in one, and the more complex vegetable sugars up to starch and wood possess molecules that are veritable documents, with hundreds of these "words" linked end to end. The ivy leaf or pond scum knows nothing of such verbose starch molecules, but builds them with fine craftsmanship from the crudest supplies.

Throughout history man has been impressed by the prowess of living things and their "vital activity," and hence the great importance of making these same organic compounds in the test tube. Only now that such attempts have succeeded may we say with confidence that no mysterious, *sui generis*, potent "vital force" is required for their manufacture. The plants must have remarkable tools to perform so neat a job, but that is all. And so they do have. One of the finest is this green chlorophyll. Plants that lack it cannot make their sugars and must live on others. And, conversely, cells that contain chlorophyll can still make sugar away from any plant body.

These chemical tools, richly present in all living things, perform seeming miracles beyond the rarest virtues of Aladdin's lamp. Obtained early from yeast, and bearing still the name then given them—enzyme (in yeast or leaven)—they preside over the flux of matter in living bodies and are the orchestra for the dance of life. One builds sugars; others break them down, each enzyme acting in its special way, to alcohol or the acid of sour milk or of vinegar or back to carbon dioxide and water; some form starch, some wood. Some form or destroy fats. Even proteins, bodies like gelatine or egg white, the most invariable components of protoplasm, are erected adroitly and exactly, just the right ones for each different living being.

Dissolved in the water of protoplasm are hordes of molecules of many molecular races. They dash about, collide, rebound, mostly unaltered by a momentary meeting with each other. But now and then—now and then, that is, in relation to the number of such collisions; exceedingly quickly according to our ideas of time—such close-pressed molecules exchange atoms or even combine, and new and different ones result. Chemical change occurs; new substances are formed. The enzymes control this play and largely determine which molecules react with which and what results.

Enzymes are a special group in the large and interesting class of catalysts—agents, mainly chemical substances, which are more important in our daily lives and our modern industry than any others. The action of catalysts, though only partly understood, is potent, and without them we, as living machines, could not exist, our great chemical industries would fail, our foods, drugs, dyes, fabrics would dwindle, and many of the activities that go to make up the complicated life of civilization must disappear. Catalysts guide and accelerate all types of chemical change. A simple example may help the picture.

Within a lump of iron are close-packed myriads of molecules jostling busily against their neighbors, oscillating about some

home position, going always but arriving nowhere. Outside the lump is air, whose widely separated molecules of nitrogen and of oxygen dart freely in all directions and recoil as freely from their constant collisions. Within the lump is only iron, and no change occurs; without is air, also unchanging. But at the surface, where oxygen bombards the iron, they can react, and slowly iron changes to rust or iron oxide as iron and oxygen molecules combine. If the tempo of the movements is increased by heat, rusting goes faster. Powdered iron tossed through a flame rusts, or burns, so rapidly in air that the falling grains sparkle brightly. And cold, of course, prevents the reaction.

Apparently here the simplest kind of molecular change occurs. Yet how misleading; for dry iron will not rust in dry air. Iron and oxygen combine only when water molecules are also present, though these remain as water at the end. Lilliputian preachers, these, uniting other molecules, entering into the ceremony of the union but remaining still themselves and ready to repeat innumerable times with new couples the nuptial act. Just how even so simple a rite is carried out is not yet fully clear and is today a problem actively investigated. For this is an entirely typical case. The great bulk of chemical changes, even the simplest, require some agency to help them take place. Such agents, which hasten action, are the catalysts.

Heat is a universal one, water hardly less so. Light is often most powerful—a glass bulb filled with hydrogen and chlorine is harmless in the dark, but tossed into the sunshine it explodes before it hits the ground. Acids, lye, salts, a great variety of substances catalyze chemical reactions. Some are catholic in their influence; others very limited. The enzymes are a special class. The aristocrats of skilled workmen, each one does one or a few jobs only but performs these with superlative dispatch. Many reactions that they carry on are also catalyzed by other agents, but not so exactly or so fast. At least three enzymes in the stomach and intestines cooperate to digest a piece of meat

in four or five hours. Strong acid will do the same, at body temperature, only in many weeks and then part of the meat is completely lost as food. Still, the essential difference is not of kind but of excellence.

From the One, Many

Further, catalysts may lead to their own formation—auto-catalysts these are. Consider a candle standing on the table, maybe for months. Its smooth wax remains as sleek, its wick as stiff and white, as when it was placed there. Yet wax does combine with oxygen and burns and gives off heat. Light a match and touch the hot flame to the wick. It glows a moment, falters, then from the wick grows out another flame. Heat is the catalyst. The match is no longer needed; its heat started the combination of wax and air which gives more heat and more burning. From the catalyzed reaction grows more catalyst.

Or consider a brick of butter, fresh today. Tomorrow still quite fresh and next day palatable but possibly with just a trace of rancid smell. Another day it hardly does for cooking, and soon it is completely spoiled. If the amount of rancidness be measured, the results bear out experience, for it increases very slowly at the start, then faster and increasingly fast. The butter fats are broken down as it spoils and ill-tasting acids result. Yet the change is not spontaneous; a catalyst is needed, and acid serves as one. Hardly any is present in fresh butter and spoil-ing is very slow. But each bit changed forms more acid and so more catalyst and so leads to faster change.

And similarly, once the first few molecules of enzymes had appeared, it is fairly easy to account for their continued exist-ence and increase. Many of them are proteins; some are per-haps simpler substances. If they direct the flux of synthesis and by their action cause new molecules to form—the sugars and the proteins and all the rare content of protoplasm—why not produce themselves? They must; they do. When one yeast

plant (containing just one unit of organized protoplasm, just one cell) divides in two, each daughter cell in two, and these again, and so on until a flourishing colony of progeny exists, the quantity of enzyme present multiplies apace. Each descendant has the same supply as did its single ancestor. The process of autocatalysis is at work here. Just as acid in butter leads to the production of more and more acid, so an enzyme may lead to the production of more and more of itself.

One can almost watch an enzyme grow by doing the simplest experiment. Place some crushed meat, or almost any foodstuff, in a little water in a small flask. Connect its opening with a long fine tube held horizontally and stopped at its far end with a drop of water. Keep the flask warm, about body temperature, and then observe it at intervals, say every hour. What occurs? The meat is largely protein and fat, the air (and water) about it contains oxygen; and the story of the candle repeats itself. At first
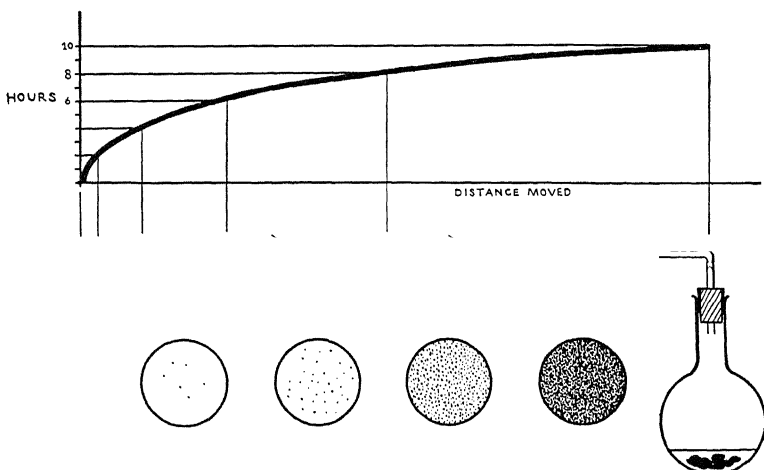

Fig. 7. Bacteria in a flask containing warm meat mash increase more and more rapidly. Their enzymes cause an accelerating disappearance of oxygen, to burn the foodstuff; and the index drop in the horizontal tube moves with increasing velocity hour after hour. The distance moved during a given time interval, say two hours, doubles with each succeeding interval. The circles below indicate the number of bacteria to be seen in a microscope field after various times.

no stuff is oxidized, a catalyst is lacking, and the drop of water remains at rest in the end of the tube. In two or three hours it begins to travel slowly toward the flask, measuring, by the distance moved, the oxygen that has disappeared from the air. It crawls a while, so slowly that only at long intervals can a change be noted. But the intervals shorten, the change increases, and after half a day the drop slides quietly along before one's eyes. Oxygen is disappearing at increasing speed, protein is being burned more and more rapidly; a progressively larger quantity of catalyst must be present. What is it and how formed?

Why, simple enough, bacteria are growing. A few are present at the start, being everywhere; and finding food and water, oxygen and warmth, they multiply. One forms two, then four, then eight, doubling the number two to three times every hour, and in the day billions have formed. The water comes to teem with them, as any microscope will show. But if the meat and flask were sterilized at first by boiling, no germs appear—and then the drop remains static at the end of the tube. Each tiny bacterium contains enzymes that catalyze the oxidation of the meat; each division into two also doubles the enzyme present. Organisms growing, enzymes forming themselves—the two are very similar; so like indeed that cases have appeared which have been very difficult to classify.

It might seem strange at first to find that entities exist as to the nature of which, alive or not, fierce controversies rage in scientific groups. There are many such, dozens of viruses alone. A man takes smallpox. His fever mounts, his pulse runs fast, pains play along his back, his skin erupts in pustules. Smallpox is a particular disease, different from every other, documented by its own distinctive signs. True, the changes seen are in a human body, but since these are a unique response to some condition, they serve to identify the disease. Some definite thing is happening in the smallpox patient. Further, the causative agent is not limited to this one body but

is transmitted with the greatest ease to others; the disease is infectious. A minute bit of mucus from the mouth, even a light contact with the skin of the sufferer or with something he has touched, suffices to carry contagion to a healthy man. Some substance has to be conveyed—infection does not pass through walls or rubber gloves—a substance completely destroyed by boiling. And after this inoculum has reached the second person it must grow. No signs of disease appear for many days. Then they become increasingly severe. Then, and not before, this second person's body dust can carry farther the disease. The bit of smallpox virus grows in a man just as the typhoid bacilli grow in the jelly and, after growth, can be carried on time and again to fresh colonies.

Why doubt then if the virus is alive? For one thing, it is small beyond the most searching microscope; no one has even seen its body. Fluids from smallpox patients have been forced through layers of porcelain so finely porous that the tiniest of bacteria are held back, but the virus passes through. Again, it has no measurable life of its own; it grows only in human bodies and does not "breathe" or exhibit any activity away from them. Perhaps the human tissues are its only food; without this it rests dormant. Perhaps, though, it is just an enzyme which leads to chemical reactions in the protoplasm of man, from which are formed more of this enzyme as well as poisons that produce the symptoms. Truly the question verges on the meaningless, for there must be in any case some complicated enzyme molecules forming a minute "body." Now does this "live" or not? Categories can be very useful, but the boundary walls must not be built too high or too rigid.

"Before I built a wall I'd ask to know what I was walling in
    or walling out, and to whom I was like to give offence.
Something there is that doesn't love a wall."

It is no accident of poetic fancy that the phrase, "The flame of life," is common currency. A living organism and a flame

are running systems, in more or less perfect equilibrium, through which flow streams of matter and energy undergoing transformation. Each is sensitive to change in its surroundings and responds to them, each has a structural organization, though here the most apparent difference between the two lies; most of all, each creates itself and grows. It is almost or quite impossible to describe living things in terms which do not describe the flame, except by recourse to such secondary differences as the actual temperature of existence or the absence of a watery menstruum. Both are autocatalytic systems with catalysts or enzymes that produce themselves and the other ancillary substances that form the matrix of their being.

Given, then, the proper enzymes, acting upon surrounding water and dissolved substances, there would grow about this enzyme nucleus the substances of protoplasm. There would be sugar formed, to serve for food and structure; protein would appear and give the forming cell its unique characters and special composition; fats would arise, to act as a further store of food and, more important, help to isolate and protect the fragile mass from its environment, weaving about it, together with the proteins, a fragile veil, yet firm enough to repel the bombardment of vagrant molecules and resist the sway of water currents. For fats spread in thin films over water, soaps (from fats) form foams and bubbles, proteins set into jellied masses; and as these substances appear by the action of enzymes they would tend to gather into films and meshworks and form a fairly definite structure. So the new substances would build their edifices; surrounded by a film secluding, protecting, and restraining them—there would a cell be born. Now the enzymes would act undisturbed. In through the protecting wall come water and foods; out go waste products of all kinds; inside, the simple chemicals become the complicated stuff of protoplasm.

By the agency, then, of non-living enzyme molecules the substance and form of protoplasm could come into being. Once

formed, it would continue to increase, to reproduce, to guide its molecules along the ways of change and so to eat and breathe —in short to live. And from one bit of protoplasmic seed the whole tree of living forms could then evolve. Into the living loom pour simple crudes—water, carbonic acid, ammonia, and some salts—there to be whirred through the enzyme machine, drawn into threads of protein and woven at last into the beautiful patterns of a living tapestry. A magic cloth, indeed, is this that spins itself. Protoplasm forms more protoplasm; living things beget themselves. A Moloch, life, consuming matter in its path; yet fashioning from it living stuff, more and ever more while the supply of matter lasts.

All this, once the enzymes are on hand. Yet how did these first enzymes, the artificers of protoplasm, come into being without the aid of antecedent enzymes? This is perhaps the hardest gap of all to bridge, but not impossible. We cannot positively know how they arose, but scientific rigor does not deny us the right to surmise as to how they might have arisen. The possibilities are many but here can only be hinted at. Many substances that might easily serve as intermediate building stones are known to form. Carbon, hydrogen, oxygen, and nitrogen are probably present in all enzymes, iron in many, phosphorus, sulphur, and magnesium in some. Heat alone causes several of these to combine into simple molecules. The energy of sunlight leads to the formation of other stuffs. Flashes of lightning are able to induce important chemical combinations, especially the "fixing" of the nitrogen of air—the prototype of one of man's methods. Many of these simpler molecules are very active and rapidly combine with each other. Some of the new ones catalyze the formation of themselves and others. All manner of atomic edifices would result from these influences, randomly thrown together in every pattern. More and more complicated units would be fashioned, decomposed again, altered, built anew. Always motion, always reaction; a seething, restless horde of molecules, colliding, exchanging atoms, chancing on

new combinations, rushing to other contacts, always changing.
And by chance, at last, an autocatalytic enzyme molecule ap-
pears. Unlikely? Most unlikely, almost impossible. Yet what
the precise tools of the ivy accomplish in minutes man dupli-
cates with his crude chemical implements in months, and
chance and change might do the same in years---in billions of
them.

Somehow life arose, and now on earth each tiny cell enacts
its deathless drama. In every little theatre of life the scenery
is set, the players take their roles, the plot unfolds. We have no
program to this play; many of the actors are unknown and
· regions of the stage are quite obscured. But it has brought real
pleasure to the audience peering at it through microscopes and
colorimeters and may intrigue many others to attend when they
have found the way.

Carbon Dioxide

Oxygen
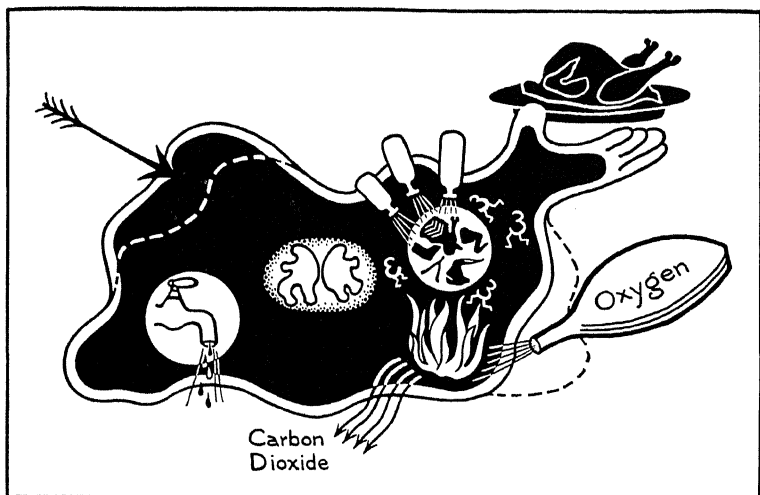
Chapter Two: Odyssey of an Amœba—
Simple Cell Life

Focus a microscope on a drop of blood obtained from a finger prick. The color disappears and the opaque fluid becomes a crowd of disc-like bodies, pale orange, piled in irregular masses or floating singly in a colorless liquid. Smooth, round, flat, these "red" corpuscles of the blood are powerless barges jostled about by the currents in the plasma sea. Present in enormous numbers, vital to our life, they do not now concern us except as they obscure their paler fellows. The white cells (leucocytes) will be overlooked without a search, for they are rare, a scant seven thousand in a cubic millimeter or one hundred million odd in a cubic inch, compared with seven hundred times as many reds. They will be found more easily in pus, which is made largely of their bodies. But here comes one into view; not floating freely, but clinging to the surface of the glass plate on which the drop is placed. The leucocyte's bulging outline is sprawled out, with several protuberances stretching away from the main mass. Within this margin is a clutter of

many small grains that move irregularly and one larger lump
that seems at rest. The whole is not half of one-thousandth of
an inch across, yet its parts are clearly visible.

Not much appears to go on unless the white cell is kept
warm, but a light bulb placed close to the microscope supplies
adequate heat. Now the leucocyte "comes to life." One of the
bulges (really "false foot" or "pseudopod") stretches out, the
content of the cell flows into it, the main mass squeezes in
behind and soon the peninsula contains the whole mass.
Another bulge appears, again the protoplasm crowds into it.
The leucocyte is slowly crawling on the glass. Soon it is lost
to view beneath the red corpuscles.

It is not difficult to obtain the white cells separated from the
others and immersed in plasma, the fluid part of blood. Let us
take such a drop of plasma with leucocytes floating in it, add
some typhoid bacilli scraped from a mass growing on jellied
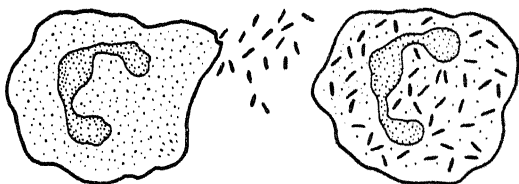
FIG. 8.   Leucocyte at start and at end of engulfing typhoid bacilli.

agar, mix them well and keep the whole warm. After some
time the bacilli are seen, of course through the microscope, to
be within the cells. It matters little if the typhoid germs be alive
or previously cooked to death, the leucocyte devours them with
equal ease. Often, a hundred of the tiny sausages make a com-
fortable meal for one white cell. True, if the germs are alive to
start with they remain alive for some time after ingestion, and
it may fare ill with the white whale carrying an overload of
Jonahs. Within the body, the leucocyte's life is of little import
if it but discharge its duties of defending against bacteria and
helping to clear up all sorts of tiny particles of debris. But we

are interested in the leucocyte as a tiny living animal, one, in fact, closely related, in much that it does and is, to the familiar amœba of pond water. Bacteria or any other tiny bits of living or formerly living matter are just so many food morsels to be eaten, digested, and used for the needs of the cell.

We can spy on the leucocyte, or more easily the amœba, obtaining its meal. Here is a small amœba crawling slowly along the glass slide by means of its pseudopods. In one direction lies a tiny green filamentous plant, a string of protoplasmic masses enclosed in "wooden" boxes. The amœba oozes here and there, without seeming to make a course for anywhere in particular; yet fairly soon it somehow manages to reach the thread. Is this pure luck; or the principle that by moving about at random for sufficient time one must cover the available territory and run into whatever is in it; or is the amœba somehow guided to the food? With patience, we could settle this by watching one amœba after another and carefully noting the course each one takes, but it is much simpler to place a large
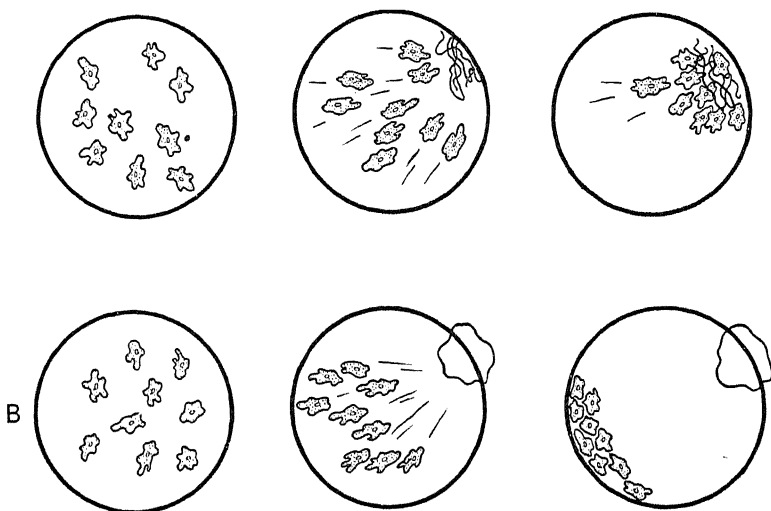
FIG. 9. Amœbas gathering about food, algæ (A), and avoiding injur (B). Movements are directed by the concentration of chemicals.
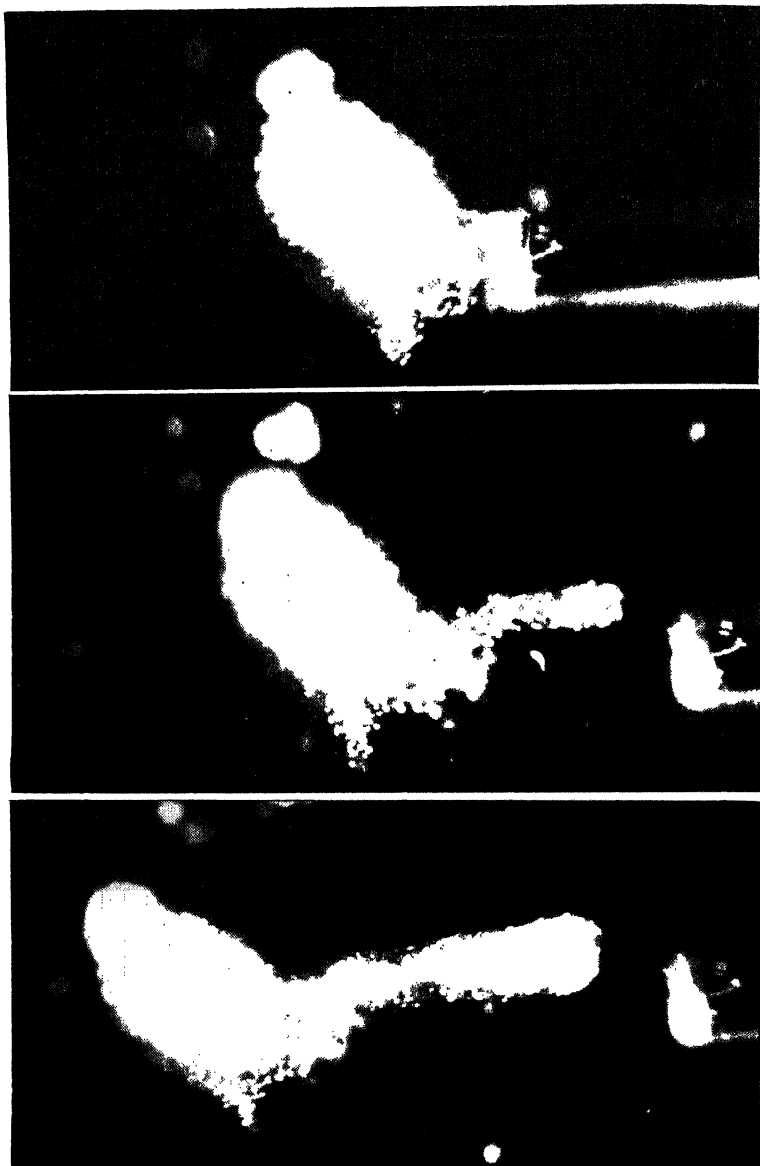
FIG. 10. An amœba, stimulated by the poke of a glass needle, "flows" away from the region of disturbance. (Microphotographs from the film, *The Nervous System*, by Gerard.)

number of amoebae at random in a large drop and then intro-
duce some food material near one edge. We need not watch
long. Almost at once a population drift toward the food is
present and soon the whole amoeba mob is clustered densely
around the plants. Clearly they have somehow been directed,
and since there is every reason to doubt their possession of any
means of seeing, smelling, or otherwise becoming "aware" of
the object, the cue must be so simple and direct that it is almost
compelling.

The little beasts are "irritable" in the sense that environmental
differences make them move toward one condition or from
another. In this case, minute amounts of chemical substances
which are produced by the plants and spread through the water
serve to direct the amoebae. If, instead of the plants themselves,
a bit of juice squeezed from them, or even water in which they
have been kept, is placed somewhere in the amoebae's pool,
these hungry mites will still gather in this region. Other sub-
stances, such as weak vinegar, will similarly drive the animal-
cules away from them; which is a very useful response, since
vinegar would soon kill the cells if they remained. But we dare
not conclude from this that the amoebae are being intelligent,
for a moderate electric current, which is just as fatal as the
vinegar, draws the amoebae along it before exploding them.

No, all these substances force the movements of the animal,
in part, by making that part of its surface which is nearest to
them more, or less, tense than the opposite one. If the surface
facing the vinegar becomes more tense than the far one—if,
that is, its surface tension increases—then the bulk of the fluid
inside is forced from this side to bulge out the other and the
whole drop will move away. The reverse occurs in the presence
of substances from food, for these make the near surface give
way, lower its surface tension, and the amoeba advances to its
meal. In the same way, a drop of alcohol on a sheet of glass
moves toward a warmed region while a drop of oil moves from
it. A simple mechanism, this, for getting the amoeba into desir-
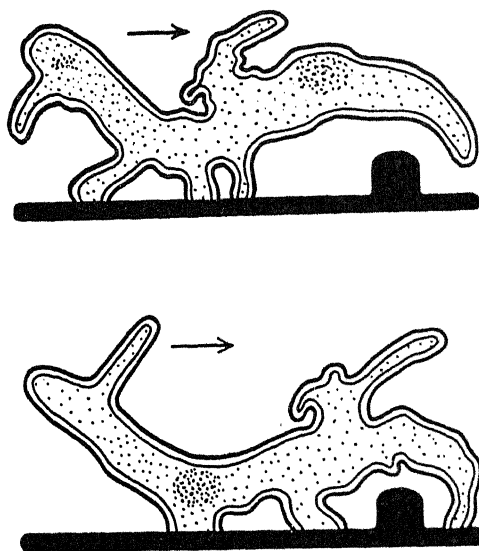
FIG. 11. The amœba's movements are not so simple, either. Here is one "stepping" over an obstacle.

able and out of dangerous places (for an amoeba would ordinarily never come into the presence of considerable currents, though it might easily encounter the vinegar); yet perhaps not so different from the equally automatic devices that make a man sneeze away an irritant that tickles a hair in his nostril. And, in truth, not so simple either, as we discover on studying our animal with even finer tools than the microscope.

We return to our peep-hole just in time to see the hungry amoeba, which has by now sprawled itself over to its prospective meal, give an amazing performance of sword-swallowing. There is no mouth, of course, or any ready-made stomach into which to swallow the plant; for that matter, the thread may be many times as long as its captor. No matter. The protoplasmic blob reaches and flows around an end of the woody filament and soon surrounds it like a lollypop on a stick. Then, as the amoeba crawls still farther, the thread bends, coils on itself, and finally becomes wound up tightly within its living sarcophagus.

Nothing more seems to happen, and we might imagine the little beast sleeping peacefully after a large dinner. Really the work is just beginning, for the amœba has only ingested its meal and has yet to digest it.

While the amoeba now remains still, we can turn the high power of the microscope upon its fairly transparent body and watch this process of digestion. Possibly, when the alga thread was engulfed, a bit of water was included with it, but whether this was so or not, the coiled strand is soon seen to be clearly separated from the amoeba's protoplasm. It lies sus-

FIG. 12. Amœba eating a filament of algæ (above), and a chloroform drop "eating" a thread of shellac. The visible events look very similar. (Partly after Rhumbler.)

pended in a tiny, perfectly clear droplet of fluid, a vacuole, the walls of which are just the protoplasm of the amoeba, even though they were originally from the slightly thickened protoplasmic surface in contact with outside fluids. The vacuole develops around the food mass wherever that may happen to lodge and not at some prearranged portion of the tiny anatomy. This simple arrangement is all the stomach the amoeba has with which to work.

We continue to watch but see no further movements; the walls of the vacuole do not churn or grind and so mechanically break up the food, as do the muscular walls of more complicated digestive systems; nothing at all seems to be happening. Yet, slowly, changes are occurring, even visibly, for the sharply outlined green algal spiral becomes blurred, the green disappears, the fine granules and rods present within its protoplasm smudge and vanish, and eventually nothing is left of the plant cells but their thin, tough walls of wood. This undigestible and useless
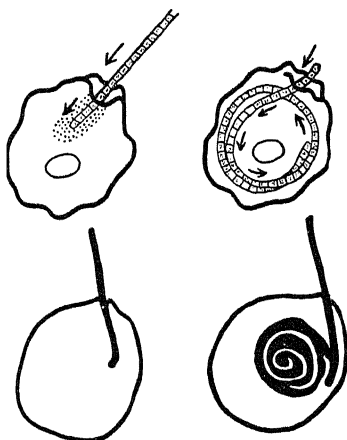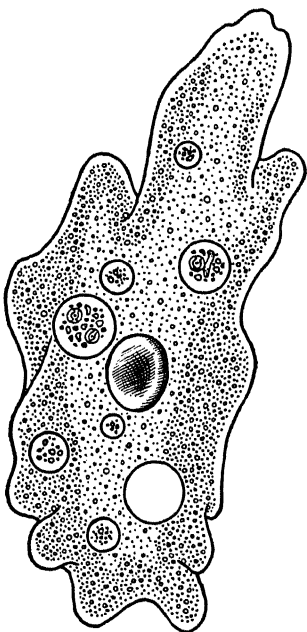
FIG. 13. Amœba, with several food vacuoles and a clear contractile vacuole. The food is in several stages of disintegration in various vacuoles.

carcass is finally eliminated from the amoeba by the same straight-forward technique which led originally to its ingestion. The amoeba's protoplasm flows forward until the residue comes to lie against the hindmost surface, which opens and returns it to the great outside, while the amoeba sluggishly goes on its way.

Somehow, while it remained in the vacuole, food was digested and the useful substances resulting from this were absorbed or taken into the protoplasm of the amoeba. Yet we saw no movement during this entire time. Clearly the vacuole fluid was vastly different from the original pond water in which the algœ far from disintegrating, grew and flourished. Into the vacuole stomach must have come from the amoeba's protoplasm, along with the water, some dissolved substances able to act upon and digest the protoplasmic materials of the algœ. Such digestive juices are present in all stomachs, and when large quantities are available, as, for example, in our own, it is no great problem to collect enough juice for study in the test tube. And, right enough, if a piece of meat or a bit of hard-boiled egg is placed in this gastric juice, it is quickly seen that the food softens, breaks up, and finally disappears, while the originally clear liquid becomes for a time cloudy and opalescent before it again turns perfectly transparent. During the same time, like bits of food placed in an equal amount of water remain solidly unchanged.

Of these chemical agents, the enzymes, which preside over important reactions, such as the digestion of foods, we must take account later on in some detail. But now we have satisfied ourselves that somehow the complex insoluble substances present in the food have been torn apart, chemically dismembered, and reduced to smaller, simpler, soluble substances, which, like sugar dissolved in water, are invisible, and which, by passing easily along with the water molecules, can diffuse out from the vacuole into the mass of protoplasm, which is the amoeba's body. This is the true importance of the whole complex process of finding, eating, and digesting a meal; to bring these dissolved molecules, the fragments of food, into the protoplasm of the cell. It is here that they become useful to the animal. And it seems likely enough that the substances produced by breakdown of the alga's protoplasm could be effectively used in building up that of the amoeba. But even now, when the amoeba has not merely digested its food but even disposed of the detritus, the work is really only well begun.

In the body protoplasm, of the single cell which constitutes an amoeba or of the myriad cells which make up a man, a cold flame is steadily burning. The chemical turmoil and traffic called metabolism somehow leaves the main characteristics of the protoplasm in which it occurs essentially constant. Of course, stimuli produce temporary changes, but the system always reverts toward some steady state, much as a candle flame flickers in a passing draft but regains its even glow when the air subsides. In the candle flame the metabolism is destructive; the candle fats are burned by oxygen to carbonic acid, and the candle slowly disappears. In protoplasm, likewise, fats and sugars and albumin are also being constantly burned away. Strangely though, the animate candle here does not shrink, but grows. Not only are the combustible elements of food and protoplasm being continuously destroyed by disruptive metabolism, catabolism, but more rapidly than this the protoplasm is being rebuilt by constructive metabolism, anabolism, from a

continuous new supply of food. Stop the food and the amoeba, like man, will starve to death, while slowly burning up its own body to keep going.

This is reminiscent of the famous race, almost a half-century ago, of the Hudson River steamers, the *Oregon* and the *C. Vanderbilt*. The *Oregon*, with fires blazing and paddles churning, entered the last lap of the race well ahead of its rival but with no more fuel. The wooden furniture, berths, all dispensable parts of its structure, were torn up and fed to the furnace until the emaciated hulk paddled victorious into the harbor off the Battery in New York City. So the starving amoeba, whose protoplasm contains much the same material that is present in its food, burns its decks and finally its very hull, and is gone. But again the comparison with man-made machines, as with candles, breaks down, for the protoplasm continually rebuilds itself. Repair not only keeps pace with destruction but, during much of the time in most living things, building is more rapid than wear, and growth results. The story of how the food fragments which diffused into the protoplasm from the vacuole are swept into the chemical dance, passed along a reception line of enzymes, and eventually assimilated into the society of molecules which are protoplasm must be told in more detail later. But just this assimilation is the crux of the whole activity of the amoeba and of all other creatures.

Most of this subtle alchemy is quite invisible and must be unraveled indirectly; but its consequences can be seen just as clearly under the microscope as was the earlier hegira of the hungry amoeba in search of a dinner. We have watched this animalcule, only a hundred-fiftieth of an inch long, crawl to food, engulf particles, digest them, extrude their solid residue, and go its way seemingly quite unchanged. But if we measure the cell from time to time, a slow growth in size is readily detected. When it has almost doubled its volume (not its length) there occurs a series of bizarre events indeed. To do them justice, they must really be followed under the microscope or, even better, seen in speeded-up motion pictures taken through a microscope; for words convey but little of the glory of a sun-

rise, and here is one in miniature. The delicate, intricate, and entirely regular maneuvers through which all elements of the cell pass in changing from one large parent amoeba to two small daughter ones constitute one of the grand strategies of life. Practically all living cells that grow and reproduce follow this general pattern of cell division, mitosis (forming threads), with unbelievable faithfulness.

First the nucleus, which was a clear, practically structureless fluid droplet, separated from the surrounding cell protoplasm by a distinct membrane, begins to cloud up. The cloud condenses slowly and takes the form of a much-entwined thread of more solid material, called chromatin, while the membrane around becomes blurred and broken and finally disappears. While these changes within the nucleus are progressing, a tiny granule at one side, tiny even compared to the nucleus, divides and the two halves move so as to occupy opposite poles of a diameter through the nucleus. Beginning as they move and continuing after they are in their new positions, there grow mysteriously from them as centers, two rosettes of gossamer threads that radiate in all directions to the farthest limits of the cell. At this stage the picture is like that of a Christmas card showing beams of light radiating from a star. By the time these two "asters" (stars, as they are appropriately called) have fully formed, the nuclear membrane is entirely gone (amoeba is rather nonconformist here) and the chromatin thread has shortened and thickened and divided into some determined number of stubby bars (chromosomes) which line up in the equator
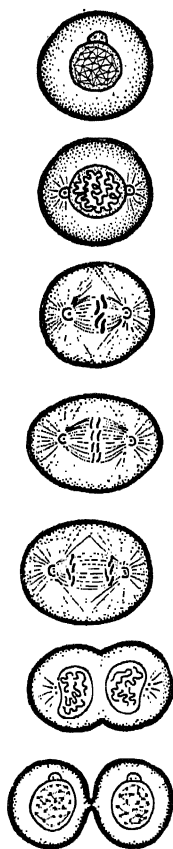
FIG. 14. From above to below, successive stages in a typical cell division by mitosis. The really crucial step is from the third to the fourth stage, when each of the chromosomes lined up on the spindle divides into two equal halves.
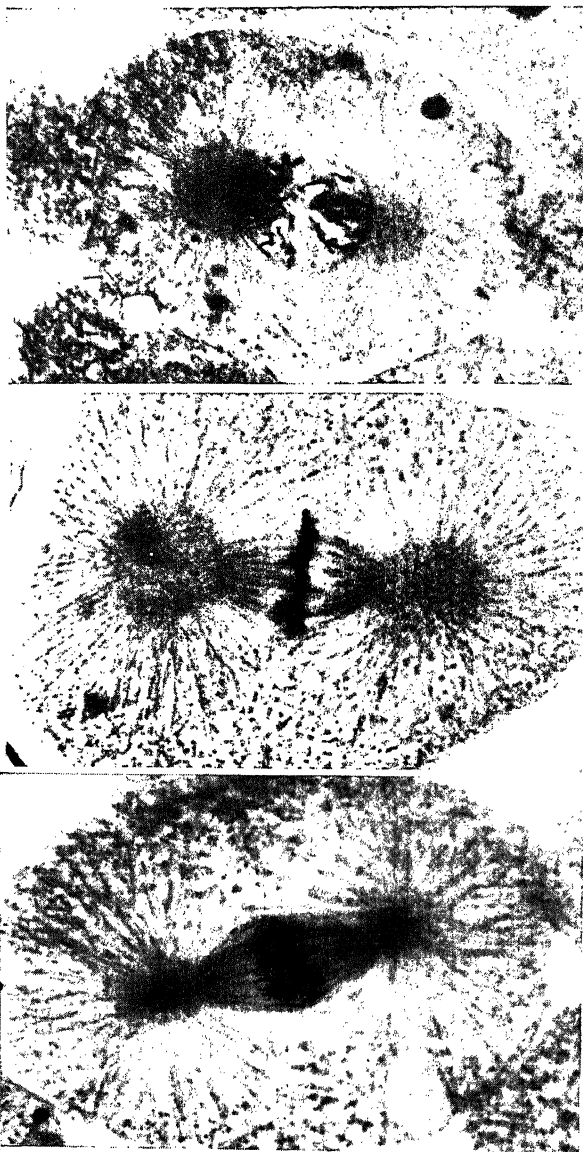
Fig. 15.   Three stages in the mitosis of a fish egg (whitefish). Microphotographs at an enlargement of about 700 times. A, chromosomes and asters forming. B, chromosomes lined up and about to divide. Spindle and asters are well developed. C, chromosomes all divided and moving toward poles of spindle.

between the asters. The rays pointed toward them become more prominent and gather together so that a clear spindle is seen.

One might say that up until now there is still one cell; the next step is definitely over the line, for now each chromosome separates along its length into two exactly equal halves. "Exactly" is true to an unbelievable degree, for the daughter cells in turn divide, their offspring do likewise, and so on; yet, the chromosome halves that are formed during each division remain, generation after generation, exactly equal. One-half of each chromosome now rapidly moves toward its own end of the spindle, leaving the center more or less empty. Then the whole process unwinds itself; the rays of the asters shrink and disappear, each group of chromosomes stretches again into a contorted thread, which becomes longer and thinner until it breaks again into a cloudy mass, and a new membrane forms around it. Meanwhile the cell surface becomes indented along the circumference midway between these two nuclei, and the cleft becomes ever deeper until the whole mass of protoplasm has been pinched in two. The two daughter amoebae separate and wend their individual ways in search of another meal.

Somehow, out of the foods, protoplasm has built itself. Somehow, all the individual structures, substances, and parts of the cell have increased and multiplied. Somehow, one individual has become two and like has produced like. How these marvelous chemical and architectural feats are achieved constitutes a basic problem of the science of life and one to which there are but partial answers. To explore these, we must for a time resign our post at the microscope and study protoplasm by other means. We may be confident, however, that so long as we keep the amoebae in enough fluid with food and oxygen available, generation after generation of them will live out their simple lives—laboriously crawling away from danger and toward food, ingesting and digesting their tiny meals, stoking the chemical flame of life, excreting the waste, growing to maturity, and finally reproducing in kind a new generation to repeat the cycle.
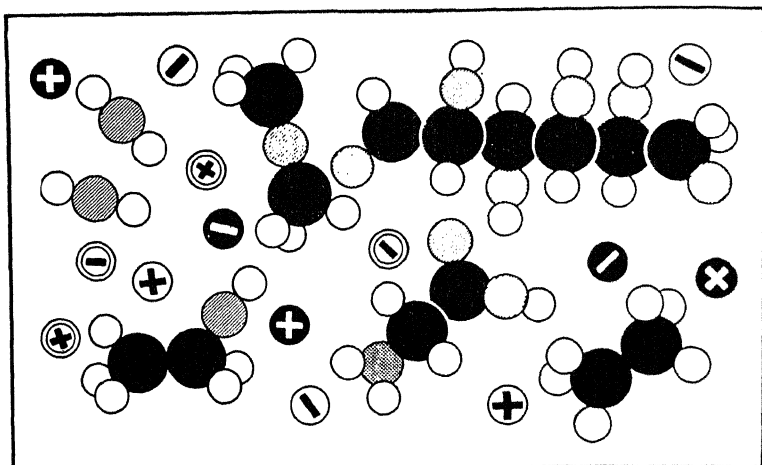
Chapter Three: Cell Substances—
Protoplasmic Chemistry

Life, we say, is the most valuable thing on earth—and so it is. True, we usually think first of our own lives or those of others dear to us, then of our group, our entire race, all humanity, and even our domestic or pet animals when we say this. What we mean by "valuable" is not very clear in our minds. Yet, in several senses of the word, living things are certainly the most precious objects in existence, quite aside from any anthropocentric point of view.

In their ability to do work, to transform the ordinary kinds of energy, living things excel all others, weight for weight, except possibly some of the machines made by them. When judged on a strictly æsthetic basis, in terms of what they are rather than what they do, the disparity between living and non-living is even greater. No one can really observe the elaborate structure and intricate activity of a fly or even a windblown dandelion, to say nothing of man himself or his pet dog, without a certain awe and admiration for its perfection.

In the inanimate world, our criteria of value (which can, if

you will, be translated into plain monetary terms) are not dissimilar. The simplest judgment is based on sheer bulk: two tons of iron are twice as valuable as one. One ounce of gold is sixteen times as expensive as one of silver, largely because there is much less total bulk of gold available. Even at this simple level, however, a certain qualitative difference enters, depending on the properties of different substances; steel is more valuable for strength, gold for durability.

A much more important value enters in, however, when we leave the crude materials and consider objects made from them. A chair is more valuable than the weight of lumber it contains, a fabric than its wool, or even a spool of copper wire than its weight in metal. These objects cost something to make and therefore fetch a higher price. Yet the increased value is not absorbed in the history of such an object but remains intrinsic to it. In each case the final object differs from the crude material in that it has been given a particular shape, structure or, broadly speaking, organization. This is relatively simple in the copper wire, somewhat more complex in the cloth, still more so in the chair.

Again in each case there is possible a whole hierarchy of further construction, of more intricate organization, of increased value. The copper wire can be incorporated into a fly-swatter, a motor armature, or a radio set, and in each form it is more valuable. A wool cloth with a complex pattern of weave and variegated colored threads and further built into a suit of clothes has again acquired real additional worth. A simple boxlike chair may greatly increase in cost and beauty alike by having its primitive lines modulated into curves and bends and its surfaces carved. When a block of marble is hewn and chiseled into an Aphrodite and great messy spludges of colored oil and pigment are spread upon a canvas to make a Mona Lisa, it is only an enhancement of organization that is achieved. To be sure, the aesthetic value of such a statue or picture is more subtle than the number of planes and curves and angles or of
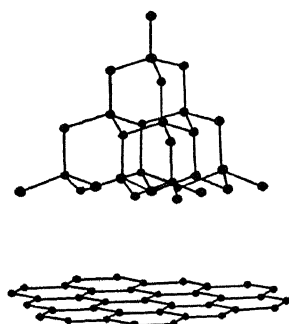
FIG. 16. Carbon atoms in a diamond (above) are arranged in perfect symmetry (the three-dimensional tetrahedron). In coal or graphite (below) they are less perfectly arranged.

colors and lines that give complexity. The added ingredient of composition or harmony, though almost indefinable, is very real.

Still another clearly recognized type of complexity follows the same rules of value. In this case, the complexity is not in a large bulk of molecules arranged to make gross patterns but in the architecture of the molecules themselves. Coal is made of carbon, diamonds likewise; but the carbon atoms are arranged in perfect patterns in the latter only. Carbon in coal, hydrogen in water, oxygen and nitrogen in the air are plentiful, simple and cheap. Yet these same four substances, or rather atoms of them, organized into more and more complex molecules give us ammonia and nitric acid, formaldehyde, vinegar, sugar, fat, wax, perfumes, dyes, and vitamins, in a rough order of increasing complexity and worth.

Now what is a man worth; an average man, one hundred and fifty pounds of him? Simple enough: thirty pounds of carbon, fifteen cents' worth of coal; fifteen pounds of hydrogen, mostly as water; ninety-five of oxygen and four of nitrogen, free as the air; ten pounds of calcium phosphate, thirty-two cents' worth of fertilizer; seven ounces of table salt, two cents; four ounces of sulphur and three of potassium; two drams of iron; a few copper pennies; and a dash of magnesium, iodine, bromine, boron, manganese, etc., just enough to constitute slight impurities.

In terms of the elements of which he is composed, a man is worth about a dollar; but in terms of what he can do as an energy and matter transforming machine, in terms of what he is as a mechanized *objet d'art*, man is surely the most valuable thing there is. As judged by any of our criteria, man and his

animate relatives lead the parade. In complexity or organization, in structural intricacy, in harmony and balance of pattern, living things are in a separate category from the non-living. Even in the simpler sense of molecular architecture, the living create and possess more kinds of more elaborate molecules, by far, than would exist without them. Here we find hierarchies upon hierarchies, structures built of other structures, and patterns in space and time that beggar the finest tapestry or most intricate counterpoint.

Some Familiar Substances

The special structure of living material we must consider later; let us now examine the materials of which it is built. The atoms or elements, as we have seen, are simple and familiar enough. Even the most plentiful compounds have little mystery; for between seven-tenths and nine-tenths or more of a living body is simply water, with table salt and a few other common salts dissolved in it. The remaining substances, all containing carbon combined with varying amounts of hydrogen and oxygen, and often nitrogen as well, bring us to the threshold of something new. Some carbon compounds are still quite simple—carbonic acid, with only carbon and oxygen, CO_2; and formic acid, CH_2O_2, with hydrogen added; even alcohol, C_2H_6O; or the acid of vinegar, $C_2H_4O_2$, are relatively familiar. Yet note the agency of life even in this simple list: formic acid is called so because it was first discovered in ants; and vinegar and alcohol are produced by the fermenting action respectively of bacteria and yeast. In fact, all of the huge assortment of compounds of carbon, excepting only carbon itself and possibly carbon dioxide, are known to chemists as "organic" compounds because they were originally isolated from organisms and are produced naturally only by living things. The chemistry of life is the chemistry of carbon, and the anatomy of plants and animals is based on the structure of this atom.

In the chemist's dictionary of known substances, there are

eight hundred thousand distinct kinds of molecules, of which two-thirds are carbon compounds. To be sure, many of these do not appear in nature, having been made *de novo* by the chemist. But most of the new substances are only slightly modified editions of those found originally in organic matter. Conversely, we are very far from having found and labeled, let alone identified, all the substances present in protoplasm; as witness the fact that each year the biochemical journals report an increasing number of new ones.

Through much of the history of scientific and prescientific thought, life and chemistry have been intertwined; and major philosophical battles about the essential nature of living things have been won and lost repeatedly with the advance of chemical knowledge. Living things were compounded, in the Greek mind, of fire, water, air, and earth, the "elements" of that day. Such a view was too simple and direct for the Roman philosophers who followed; and Galen devised a scheme which, enduring fifteen hundred years, explained health and disease in terms of the balance or imbalance of important fluids or humors in the body. Sadness, for example, was due to too much black bile, hence the word "melancholy" (melan . black, chole=bile). Anger or choler was another biliary disbalance, choleric persons being surcharged with the yellow variety; and, though we no longer believe it, we still say we are in a good or a bad "humor." The alchemists confused their attempts to transmute baser substances into gold and to distill the precious elixir of immortality; but in the course of their fumblings, they did succeed in making hydrochloric acid, saltpeter, and many other of the simpler compounds.

Later, as chemistry gropingly took its early steps and substance after substance was isolated, purified, and finally identified, interest still turned to the substances produced by living organisms. For living things seemed so clearly to have some supravital spirit infused into them that it was natural to look for something unique about their composition; and it was long a

fond hope, which died lingeringly, that some distinctive "living" molecule would come to light. Early success in this direction seemed presaged by the discovery, for example, in animal excretions, of substances previously quite unknown, like urea and uric acid in urine and guanidine in feces (bird guano). The vitalists then pointed with pride to these material evidences of a supravital force acting via living organisms and able to contort matter to its own ends. Such substances, they said, could be formed only with the aid of living agents—man would never make them in retorts and crucibles as he had the more drossy "inorganic" substances.

Hardly more than a hundred years ago, the advancing tide swept strongly in the other direction, for in 1826 a chemist made urea in a test tube from such a simple substance as ammonium carbonate. Success rapidly followed success, organic compounds were built by steps from inorganic ones, and the vital force went into the dust bin. The mechanists then held sway. The cell, it seemed, is merely a test tube, loaded with such a miscellany of substances that almost anything may form when they interact. The chemist would do as well when he had found out more about these substances and their properties. And in this century the boast has held amazingly, for the chemist has indeed been able to make most of the substances found in organisms. Substances of one important group alone, the proteins, have not yet been synthesized though their composition is fairly completely elucidated. If there be any chemical unique to life, if a living molecule did exist, it would surely be a protein. But even here the problem seems rather one of degree than of kind, for the protein molecule—even a simple one—is some two thousand times as heavy as that of water, and we are certainly more than one two-thousandth as far advanced in our knowledge of it.

Let us turn back, however, from the chemical aristocracy to consider a little further such humdrum things as salt and water. It does seem rather disappointing that so much of our precious

selves is most plebeian, as disappointing as to find one's flower
garden growing to dandelion. Water and dandelions are not
intriguing, but that is because they are so common. Judged on
almost any technical criterion, the dandelion stands out at the
advanced tip of plant evolution. It is so common, in fact, only
because it is so extremely successful. Water likewise holds an
astounding number of chemical records. It is in many ways
the most remarkable substance known.

Water dissolves more substances than any other solvent.
Water causes dissolved molecules to break up into fragments
(ions) to a greater extent than does any one of its competitors.
Water changes its temperature more slowly than does any
other substance as heat is added to or withdrawn from it. Each
of these superlatives is of extraordinary importance for the
maintenance of life. Chemical exchange occurs between dis-
solved molecules incomparably faster than it does between
solids, and with the utmost rapidity between ions, so that water
is one of the original and best catalysts. Further, living things,
like delicate instruments, are susceptible to rapid alteration of
their surroundings, not least to cooling off, so that a lagging of
temperature changes is of great importance.

In many additional, less familiar properties as well, water is
peculiarly adapted to be the matrix in which life processes
stage their play, and indeed it has been ingeniously argued
that "The Fitness of the Environment" (consisting largely of
water and carbonic acid), to support life is quite as remarkable
as the fitness of organisms to survive in their environment. In-
cidentally, still another almost, but not quite, unique property
of water must have been important in the history of life on this
planet. Life surely began in water and as surely would have
made scant progress if organisms were frozen in ice through
the year or even periodically. Yet if water did not expand on
freezing, but contracted in the orthodox way, ice on forming
would sink to the bottom of bodies of water and, in this pro-
tected position, would melt but slowly in the summer. A few

decades or centuries of this would leave our lakes and oceans enormous bowls of ice, certainly not very suitable for life. As it is, water contracts as it cools to about 40° F. but then begins to expand. It therefore rises when it is cooler as well as warmer and deep waters are, as a result, universally at this cool but not freezing temperature.

The dissolved salts in cells, by their mere presence, perform all manner of exciting duties which must be looked into farther on. Here we are concerned only with the importance of particular salts, or rather their constituent ions, as individual chemical entities. This is in part an old and often-told story. Manufacturers add, for example, a little sodium iodide, something like one part in five hundred, to our table salt in regions where the natural waters do not themselves contain the necessary trace of this substance; for when it is deficient in the body there results goiter, stunted and deformed growth, and even feeble-mindedness. In this case the iodine is not used directly by all the body cells, but it is needed by the thyroid gland to build a special secretion, the absence of which causes many abnormalities.

The majority of these salts must be present, and present in the right amounts within rather narrow limits, or the whole cell population of the body is disaffected. It is very easy to demonstrate this on the frog's heart, which continues its regular beat even when removed from the body. If left in the air, it gradually dries and stops beating. Should we drop it into pure water to prevent the desiccation, it stops very much sooner than before and becomes tremendously swollen. The absence of dissolved salt ions causes an excessive amount of water to enter the cells by "osmotic" action, one of the general influences of dissolved particles, the examination of which must be postponed. If the appropriate concentration of solute (dissolved particles) is present—and sugar will do as well as salt—then the heart neither dries nor bloats and can continue to beat. In sugar solution, however, the heart still is unable to carry on
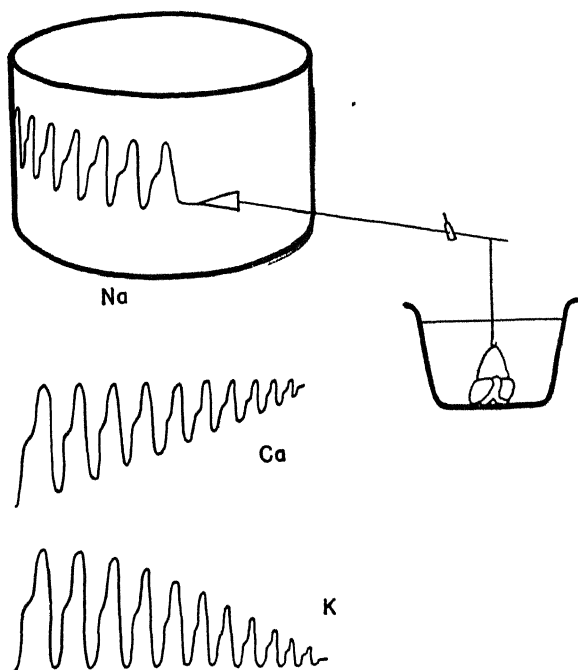
FIG. 17. The isolated frog heart continues to beat and, by pulling a light lever, marks a record on the surface of a slowly turning drum. In a sodium chloride solution of the proper concentration, the beat continues for some time, but the heart finally stops in relaxation. Adding calcium chloride may restore the beat, but the heart stops in contraction. Potassium chloride makes the heart relax and stop much quicker than does sodium. But when all salts are present in proper proportions, the heart beat continues for many hours.

more than a few minutes, though after it stops in sugar solution (but not after stopping in pure water), it can again be started if put into a solution of sodium chloride.

In this salt solution it will beat for hours, but still fails in time. This failure is also dependent on an inappropriate salt "balance," for adding a small amount of potassium chloride restores the beat, or if present at the start, prolongs the beating period. If too much of the potassium salt is present, the heart stops

promptly, completely relaxed; but even when the optimum amount is present, the heart none the less relaxes to a stop after some hours of beating. The pumping apparatus is, however, only maladjusted, not destroyed, for adding to the other salts a bit of calcium chloride yet again restores the beat. The heart, now in a solution with the right amount of sodium chloride to keep it from gaining or losing water, and about one-hundredth as much potassium and calcium chloride, can beat for days, until it has burned up the fuel present in it; and with food molecules also present in the solution, the heart may beat almost indefinitely. In the case of this organ, then, the salt ingredients of protoplasm readily show their worth.

An ordinary muscle of an arm or leg, which contracts only when messages reach it from the brain, changes, with altered salts, the ease with which it responds to such messages; in fact, if it is kept in sodium chloride solution with the other salt ingredients missing, it begins to beat spontaneously, a sort of artificial heart. The nervous system and other body parts are also overactive when calcium is diminished, as in some of the convulsions of children; and in Addison's disease, caused by destruction of the adrenal glands, potassium becomes too concentrated in the blood and general lassitude, weakness, and death in stupor result. If calcium decreases too far, as occurs when another gland, the parathyroid, is destroyed, exactly the reverse picture results. There are increasing activity, excitement, and eventually death in convulsions, very much as following strychnine poisoning. Another salt, magnesium chloride, present in still smaller amounts in the blood, though more concentrated in many of the cells, will produce deep narcosis if its concentration is increased, and it has been used for this effect in surgery and obstetrics.

Heart, muscle, brain and, no less, the amoeba and the beating cilia of the paramecium require and contain about the same amounts of the various salts. When we pass from the inside to

the outside of cells, however, this is no longer true. An ordinary amoeba gets on better in pond water than in a "balanced" salt solution, whereas its marine cousin, quite happy in the ocean, promptly dies in the pond. If as much potassium were present in the blood that bathes our own cells as is present within them, our bodies would be promptly paralyzed or killed. How a small amount of substance outside of a cell which already has a larger amount of this same substance within it can be so injurious, is a question that has to do also with the general properties of molecules and ions and the structure of cells.

The reason that protoplasm contains these particular substances in fairly constant quantity we can only guess, but one point has been often urged. Since the first living organisms, and therefore their protoplasms, very probably originated in the ocean, they might be expected to use sea water as a matrix for their own flesh; and the salt water of protoplasm is indeed rather similar to that of the sea. Be this as it may, the salts, prosaic though they seem, are hardly less essential to living bodies than the water in which they are dissolved. In the absence of water, death from thirst is a matter of minutes to days, depending on the size of the body and the rate at which water is lost. In the absence of salts, death is a matter of days to weeks, depending on the amount of storage present and the loss. In the absence of the ordinary fuel foods, man can survive for over a month and be none the worse for it; and less active or dormant animals sometimes live for years. It is not surprising, then, that animals have developed strong urges for these necessary ingredients. Thirst is far more powerful than hunger, and salt hunger can drive defenseless herbivores to the salt licks, though their carnivorous enemies await them there.

The salts of iron and copper, present in far smaller amounts than the ones already considered, are no less precious to the cell. Like iodine, these are built up into complex molecules to constitute important intracellular enzymes. One particular sub-

stance, formed in the bone marrow with the aid of iron and then carefully preserved in sealed packets, is the red hemoglobin packed in blood corpuscles.

A Touch of Chemistry

Now it is necessary to take a brief dip into the basic ideas of pure chemistry. To be sure, this is a book about living things, but these are so characteristically chemical in their structure, activity and, one might say, motif, that we cannot really think about them without some familiarity with the language of chemistry. So those not already introduced to the simple nouns and verbs of this tongue may just as well make their bowing acquaintance at this time. It saves so much laborious, wordy exposition and dull repetition to express in a few symbols, arranged in one line, ideas and happenings that might require paragraphs or pages of prose. Mathematics has been called an analytical shorthand; the words and symbols of other branches of science likewise usually express tersely and accurately the relevant facts and ideas.

Every variety of pure substance is composed of molecules which are essentially identical with one another. There are, therefore, as many kinds of molecules as there are of substances, and each kind must be as fixed and enduring in its individuality as is the substance it composes. Yet there are hundreds of thousands of different pure substances, and so of molecules, but only ninety-odd kinds of atoms from which to build them. Clearly the identity of the molecule varies with the number and kind of atoms it contains. This is readily confirmed by actual chemical analyses which do, in fact, show that any change in the kind or number of atoms results in a different molecular species.

But this is not the whole story, for, especially among organic compounds, it not infrequently turns out that molecules of two

distinctly different substances are found to contain the same kinds of atoms and in the same proportions. A molecule of alcohol (C_2H_6O) contains two atoms of carbon, six of hydrogen, and one of oxygen; but so also does the molecule of ether. Some additional factor has still not been taken into account; and difference of arrangement of the atoms within the molecule seems a likely one that has been neglected. It is, in fact, one of the great triumphs of organic chemistry that, from a study of the chemical properties of pure substances, it has been possible to deduce the precise interconnections of atoms that make even the complex molecules, and on the basis of these "structural formulæ" to build the molecules step by step as an exercise in chemical construction.

The ordinary formulæ indicate which kinds of atoms and how many of each are present in the molecule and are merely a statement of actual quantitative experimental measurements (proportion by weight of the elements which are obtained on disrupting the compound). If we inspect the formulae of a number of simple compounds, certain relations at once are apparent. Here are a few: Hydrogen gas, H_2; chlorine gas, Cl_2; hydrochloric acid, HCl; water, H_2O; carbon dioxide, CO_2; carbon tetrachloride, CCl_4; methane, CH_4; chloroform, $CHCl_3$; formaldehyde, CH_2O; ethane, C_2H_6. It is obvious that one hydrogen or chlorine can combine with only one other atom of whatever kind; one oxygen can combine with two, e.g., two hydrogens; one carbon with four hydrogens, but with only two oxygens, etc.

Hydrogen, we conclude, has a combining power or valence of one; just one "hook" on which to attach another atom. Chlorine, likewise, in the compounds indicated, has a valence of one. To oxygen, however, must be ascribed a valence of two, and to carbon of four. Nitrogen shows in its ordinary compounds a valence of three, though it can also, quite commonly, exhibit one of five. A tremendous body of chemical knowledge supports such conclusions about the valences of various atoms and, indeed, neatly accounts for their posses-

sion of just these numbers. All the known chemical elements can be satisfactorily ranged in a self-consistent table based on increasing weight of their atoms and regularly repeated cycles of valence difference from zero to seven.

We are now concerned, however, not with the basis of atomic valence, but with its consequences. In many cases, the structure of the molecule follows of necessity from these known atomic valences. Thus, in water, it is impossible that the two hydrogens are attached to one another, for the single valence of each would then be occupied, leaving no means for hooking on the oxygen. The water molecule, therefore, must be H-O-H, with each of the two oxygen valences holding one of the hydrogens. Methane, CH_4, on the same argument, must have each of the hydrogens attached to the carbon, none to each other, so that the atoms are necessarily combined together and distributed in space somewhat as follows:

$$H - \overset{\displaystyle H}{\underset{\displaystyle H}{|}} - H.$$ Chloroform, $CHCl_3$, is an entirely comparable

molecule, $\quad Cl - \overset{\displaystyle H}{\underset{\displaystyle Cl}{|}} - Cl;\quad$ and it is quite immaterial in such

a symmetrical structure whether the hydrogen is represented as attached to the top valence, as shown, or to one of the other three, for all four are identical. Ethane, C_2H_6, offers no new problem, for the only possible arrangement of atoms is:

$$H - \overset{\displaystyle H}{\underset{\displaystyle H}{|}} - \overset{\displaystyle H}{\underset{\displaystyle H}{|}} - H.$$
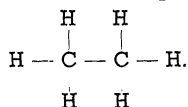
As soon as we begin to combine atoms of various valences above one, the possible arrangements become more elaborate. Carbon dioxide, CO_2, is still straightforward enough, the four valences of carbon being attached in pairs to the two oxygens to give two

C   O   N   H
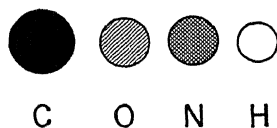
FIG. 18. Symbols for the kinds of atoms shown in molecular structures.
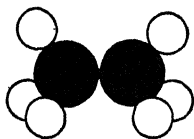
FIG. 19. A molecule of ethane.

so‑called double bonds, O=C=O. But now, of course, one of these bonds could open and still leave the oxygen and carbon firmly held together by the other. This is just what happens when carbon di-oxide is dissolved in water. The two mole-
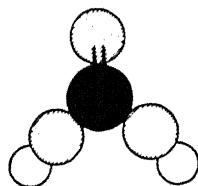
FIG. 20. A molecule of carbonic acid.

cules combine to give carbonic acid, H_2CO_3 which has the

structural formula,
$$O = C - O \begin{matrix} H \\ | \\ O \\ | \end{matrix} H.$$
The H and OH parts of the water molecule are added to the oxygen and carbon ends, respectively, of the newly opened bond.

Suppose, however, a molecule were found with the atomic formula, C_2H_6O. We know at once that all hydrogens must be attached to oxygen or carbon, which in turn must be hooked together, but we can no longer tell by inspection exactly what the arrangement is. Two structures (and only two) are possible for this molecule. If all six hydrogens are attached to carbon atoms, each carbon could hold three and its fourth valence would be left to combine with the oxygen,
$$H - \overset{\displaystyle H}{\underset{\displaystyle H}{C}} - O - \overset{\displaystyle H}{\underset{\displaystyle H}{C}} - H.$$
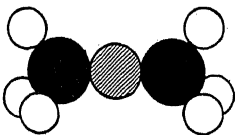
FIG. 21. A molecule of ether.

If one hydrogen is combined to oxygen, that would leave three for one carbon and two for the other, and only one oxy-gen valence would be free to combine with carbon, so that the carbons would have to be attached directly together,
$$H - \overset{\displaystyle H}{\underset{\displaystyle H}{C}} - \overset{\displaystyle H}{\underset{\displaystyle H}{C}} - O - H.$$
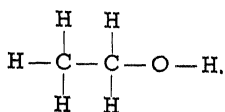
Now the fact that a certain structural formula is possible on paper does not guarantee that such a molecule can exist in reality. Many limitations restrict the conversion of blueprints into buildings. The contrary, however, is more important; it would be disastrous to our whole mental picture if two different compounds were found when only

Fig. 22. A molecule of alcohol.

one kind of molecule could be possible according to the theory. In the simple case before us, two, and only two, substances are known with the formula C_2H_6O; they are ordinary grain alcohol and the less ordinary kind of ether—methyl ether. So far, so good. Our knowledge of valence tells us that two kinds of molecules can be built with these particular atomic blocks and our experiments tell us that two do exist. But which is which? The next steps illustrate simultaneously the value of scientific theory and the crucial tests used to check it.
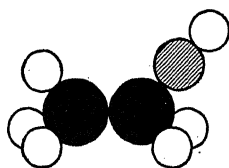
Note how the two structural formulae differ. Molecule A,

$$H-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-O-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-H, \quad \text{has all six hydrogens similarly attached}$$
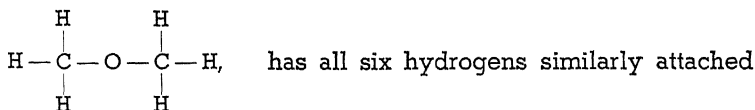
to carbons and they should, therefore, all be entirely alike in their chemical behavior; but one of the hydrogens in molecule B,

$$H-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-O-H, \quad \text{might reasonably be expected to behave}$$
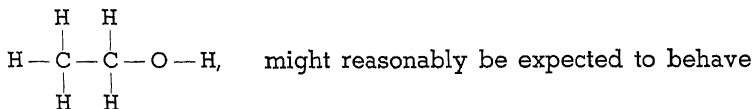
somewhat differently from its fellows, since it is distinguished from them by its attachment to oxygen. It is found, in the laboratory, that sodium will not react at all with ether, but will react rather easily with alcohol, to give the compound C_2H_5ONa, sodium alcoholate. Well and good, then, ether must be molecule A and alcohol molecule B. But on such an important point, we must be doubly sure.

The best check on this decision is actually to manufacture

the molecules. Silver oxide, Ag_2O (Ag for Argentum), must have the structure Ag-O-Ag, for silver, like hydrogen, ordinarily has a single valence. Methyl iodide, CH_3I, is, of course,

$$
\begin{array}{c}
H \\
| \\
H \quad C-I. \\
| \\
H
\end{array}
$$
When the two substances are heated together,
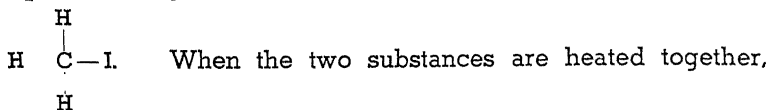
silver iodide forms as a solid, and a new organic compound is produced. Since oxygen at no time was attached to hydrogen, and since the silver and iodide, attached respectively to oxygen and to carbon, obviously released the valences of these two atoms, the reaction must have been as follows:

$$
\begin{array}{cccc}
H & & H & \\
| & & | & \\
H-C--I+Ag-\!-O-\!-Ag+I-\!-C-H & & H-\overset{}{C}-O-\overset{}{C}-H+AgI+AgI \text{ (or 2 AgI)}. \\
| & & | \quad | \\
H & & H \quad H
\end{array}
$$
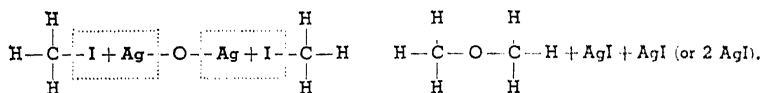
But the new compound is molecule A, which we called ether, since all its hydrogens are alike unreactive; and, indeed, ether is the substance formed.

Let us start again, with ethyl chloride, C_2H_5Cl, which must

be
$$
\begin{array}{c}
\quad H \quad H \\
\quad | \quad | \\
H-C-C-Cl, \\
\quad | \quad | \\
\quad H \quad H
\end{array}
$$
and treat it with sodium hydroxide,
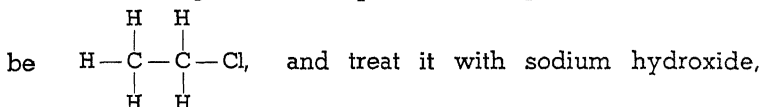
NaOH (the Latin for sodium is Natrium), or Na-O-H. There are produced the salt, sodium chloride (NaCl), and alcohol. The reaction was clearly:

$$
\begin{array}{cc}
H \quad H & H \quad H \\
| \quad | & | \quad | \\
H-C-C-\!-Cl+Na-\!-O-H\cdot & \cdot H-C-C-(\quad -H+Na--Cl \\
| \quad | & | \quad | \\
H \quad H & H \quad H
\end{array}
$$
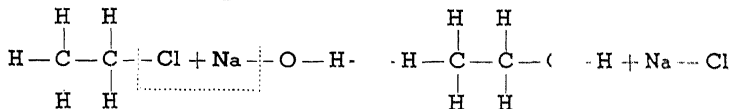
All is in order. We have made the molecule with one hydrogen on oxygen, and find that, as expected, it is the substance alcohol.

The theory is thus completely supported and, based on the insight obtained from it, it has been possible to manufacture the desired molecules. By such careful steps, building ever more complex structures from already established simpler ones,

the organic chemist has succeeded in synthesizing one after another of the labyrinthine molecules found in protoplasm. Before a successful synthesis is accomplished, the newly discovered substance must be purified, and then its structural formula established by the ways in which its molecules react and the fragments they yield when broken apart. The details of this enormous labor are beyond our interest, but this may be taken for granted: wherever a chemical formula is given, in the discussion of substances found in and produced by living things, it is the well-proved result of such indefatigable studies made upon that substance.

There! The pure chemistry is over for a while, and we can turn now to the fascinating molecules found in cells. Other than those we have already glanced at, these are all carbon compounds. But some organic molecules are small and relatively mobile and dash about in protoplasm and into and out of cells, not unlike those of salt and water. These are the small coin of body commerce. Some are fragments formed by digestion of the large food molecules and on their way into the cell to be burned up or rebuilt again into the large structural edifices which constitute the second group of organic compounds. Others are encountered rather at the far end of this continuous sequence; molecules on their way out of the cell, formed by the breakdown of various substances in it, and due to be excreted. These transient substances ordinarily are dissolved in the water of cells or in the body fluids that surround or circulate between them, and they, along with the inorganic molecules, represent the chemical milieu of life. Though highly mobile and in continuous flux, they, as well as the salts, are kept in remarkably constant balance, and a considerable increase or decrease of almost any one may be associated with profound disturbance.

The simplest and most common sugar molecule, glucose ($C_6H_{12}O_6$), one of this group, is the main immediate fuel of most cells. When too much, or particularly too little, is

present, extensive disorganization results; and in man, for
example, when the glucose in the blood is decreased by half,
stupor and violent convulsions may result. Ammonia (NH_3);
urea (CH_4ON_2); glycerine ($C_3H_8O_3$); a variety of amino acids,
related to proteins; lactic acid ($C_3H_6O_3$), originally found in
sour milk; uric acid ($C_5H_4O_3N_4$); creatine ($C_4H_9O_2N_3$), the
word means "a nitrogen-containing substance found in flesh"
(and creatine is so called because it was one of the first organic
substances obtained by extracting tissues rather than by the
more primitive analysis of the fluid or solid excretions of the
body); various types of fatty acids related to fats; and so on in
great diversity—these are the simple "extractives" of proto-
plasm.

Sugars
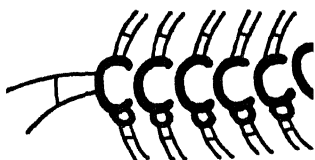
FIG. 23. "A glucose molecule is some-
thing like a centipede for all that."

Glucose, to give it its full
dignity, is in the monosaccha-
ride division of the carbohy-
drates and is one of the six-
teen possible and known al-
dose hexoses. But it is some-
thing like a centipede for all
that, with a trunk of six car-
bon atoms, jaw of oxygen, tail of hydrogen, and somewhat
lopsided paired legs attached along its length. The atoms in it
and their arrangement are shown fairly well by the following
picture, which might indeed be the "innards" of some elon-
gated bug:

$$H-\overset{\overset{\textstyle H}{|}}{\underset{\underset{\textstyle H}{|}}{C}}-\overset{\overset{\textstyle H}{|}}{\underset{\underset{\textstyle H}{|}}{C}}-\overset{\overset{\textstyle H}{|}}{\underset{\underset{\textstyle H}{|}}{C}}-\overset{\overset{\textstyle H}{|}}{\underset{\underset{\textstyle H}{|}}{C}}-\overset{\overset{\textstyle H}{|}}{\underset{\underset{\textstyle H}{|}}{C}}-\overset{\overset{\textstyle H}{|}}{C}=O$$
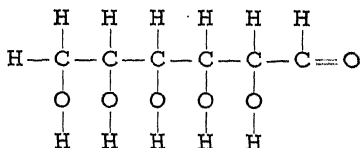
To be sure, the glucose molecule ordinarily coils itself up with

one or another foot in its mouth, not unlike a question mark; but perhaps so do centipedes. Another important similarity is that neither occupies the same position for long. The molecule, like the insect, is writhing about, twisting its atomic legs and back, bending to and fro, and occasionally opening its jaws and substituting in them another favored appendage.

Glucose is a carbohydrate because, like all its fellows, its molecule contains carbon, hydrogen, and oxygen only; and these are present in the exact proportions of one atom of carbon to one molecule of water $(CH_2O)_6$. It is a hexose, "hex" because of the six carbon atom chain, and it has cousins which are trioses, tetroses, pentoses and, as the result of man's ingenuity, septoses, etc. It is an aldose because of the oxygen jaw at one end, more professionally called the aldehyde group, $-\overset{H}{\underset{}{C}} = O;$ and the "ose," both for aldose and ketose, implies the presence of the longer legs or —O—H (hydroxyl) groups. There are other sugars, ketoses, which have that particular
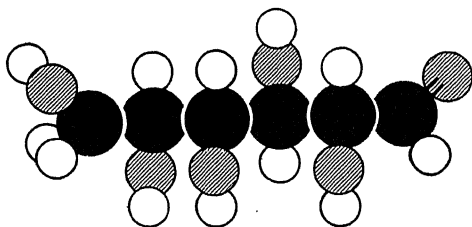
Fig. 24.   A molecule of glucose "unrolled."

oxygen which is attached to carbon by two bonds rather than by one, not at one end of the molecule but at the second or third carbon. This is the only difference between glucose, or grape sugar, and fructose, or fruit sugar; the doubly bound oxygen is attached to the first carbon (aldehyde) in glucose and to the second (ketone) in fructose. In fact, it is a relatively simple matter even in the test tube, and extremely easy for the cell, to juggle some hydrogen atoms about and change one of these into the other.

And what of the other fifteen aldose hexoses, immediate brothers and sisters of glucose? Their atoms are all connected in exactly the same way as in glucose, so that the picture of this molecule would serve as well for its fifteen sibs. They differ, not in which atoms are attached to which, but rather in which side or valence of a carbon has the —O—H attached. In contrast to the symmetry of methane, this molecule has a "front" and "back" end and the two sides are not indistinguishable. If the centipede, for example, had one pair of legs switched, so that the right one were on the left side and the left on the right at the same position on the trunk, they would be pointed oppositely in space and would considerably change the locomotion of the insect. If our right and left arms, which are "identical" but exactly opposite, were interchanged, we would have to contort some to clasp our hands. In the same way, the four middle carbons in the glucose molecule have an H group on one side and an OH on the other; and when the two are switched on any one carbon, a different molecule, with altered chemical properties, results. Two molecules are possible for attachments to the two sides of the first carbon, two for the second, and so for the third and fourth; which makes 2 X 2 X 2 X 2, or 16 possible spatial varieties (stereoisomers). Only three of these possible aldose hexoses seem to be formed in nature—glucose, mannose and galactose. The story of the remainder is a large chapter in one of the really great epochs of chemistry.

When the theory of stereoisomerism was propounded, late last century, it followed, along the line of argument given above, that there should be sixteen aldose hexoses. Yet nature supplied less than one-fifth of these. Either the theory was wrong and the others could exist only in the imagination of the theorists, or the theory was right and the other molecules, though possible, had simply never been formed by a conservative Mother Nature. This put it squarely up to the chemist. If he could himself make the missing molecules, he had proved

his point—and he did. One single chemist, Emil Fischer, one of those outstanding intellects that make us proud of our humanity, single-handed made the missing compounds. In doing so, he had to devise brand-new methods of chemical procedure, invent new chemical tools and reagents, perfect new ways of analysis to separate and identify substances so much alike, and even coin a new set of names and symbols to tell the chemical world about his results. At the end, the theory was thoroughly established and the missing sugars stood labeled on the laboratory shelf.

Finally, to complete our heraldic analysis of the full coat-of-arms of glucose, why is it a monosaccharide or single sugar-like molecule? Because there are large numbers of disaccharides, trisaccharides, and so on to huge polysaccharides. If the jaw of one glucose molecule should catch the foot of a second one, and then hold fast, as indeed it does, a disaccharide results. If the first glucose attaches to the second in one way, maltose, the sugar in malt, is the product; if they combine in another way, the product is a less common sugar; if glucose combines with a galactose molecule, lactose, the sugar of milk, is formed; and if with fructose the product is ordinary cane sugar, sucrose.
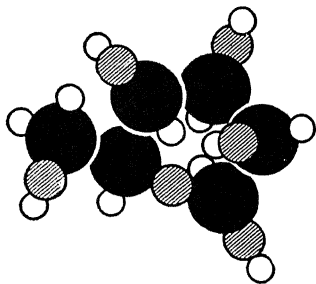
FIG. 25. A molecule of galactose has the same structure as one of glucose (see next figure) except that the positions of the H and OH on the fourth carbon are reversed. This is therefore a stereoisomer of glucose.

The two monosaccharide molecules are combined in sucrose so that their oxygen jaws are both occupied; but in maltose the aldehyde of the second glucose molecule is still free and therefore able to combine with a third molecule, this with a fourth, and so on. There seems no good reason for the process to stop until the chain of molecules is so enormous and so tangled in itself that additional
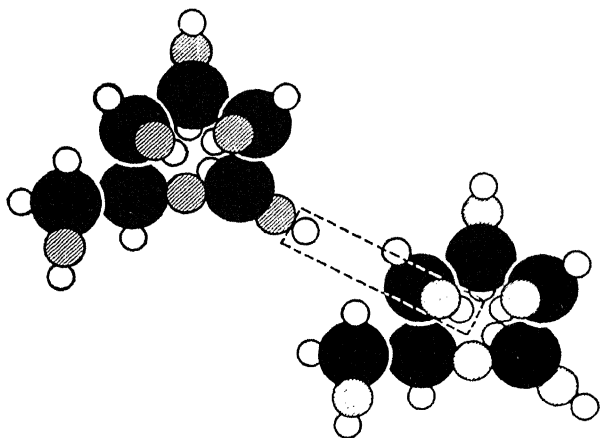
FIG. 26. Two molecules of glucose in their most stablo form. Whon they combine, with the loss of the molecule of water indicated, ono moleculo of the disaccharide maltose results. If they combined in a difforont way, tho product would be sucrose, ordinary table sugar.

individuals simply cannot hang on. We might jump from the simile of centipedes to one of a parade of elephants, the trunk of each holding the tail of his predecessor, the whole queue twisting and turning and shifting formation, yet with the whole order maintained. Several dozen glucose molecules so hung together form a simple polysaccharide, the starch of nearly all plant cells. Still larger chains form animal starch (called glycogen, since it generates glucose on breaking down) and even more complex hexoses form the cellulose of wood.

These, then, are the most common carbohydrates in the great series of polysaccharides which, as starch in plants or as glycogen in animals, are present in practically every cell and constitute its immediate food reserve. The simpler di- and trisaccharides are sometimes intermediate on the way to or from the giant starch molecules, sometimes themselves particular end products of cell action, as is the lactose formed in the gland cells of the mother's breast and destined as food for the infant. Hexose monosaccharides and their shorter relatives, especially the trioses, flit their way across the screen, some being intermediate molecules in the breakdown or building

up of glucose. But far the most important of all sugars are glucose and its alter ego, fructose, the prime currency in the chemical banking of cells.

These various carbohydrates are, in a sense, *in* but not *of* protoplasm, except for the large polysaccharides; and even starch and glycogen tend to be deposited in cells as granular solid masses, which are related to the flux of protoplasm rather as reservoirs to be tapped or replenished than as active participants. Cellulose, though forming part of the cell structure of plants, is not "alive," since the wood, even though essential to the well-being of living plants, is itself as entirely "dead" and separate as the hair and nails of mammals. But carbohydrates, especially single pentoses and hexoses combined with molecular groups of quite different species, are built into the very warp and woof of living protoplasm. Not all such compounded sugars are, to be sure; for chitin, the skeletal substance of insects and comparable to the inert cellulose skeletal material of plants, is one such carbohydrate derivative, a glucoside. Many other monosaccharides, however, are built into vastly important molecules, intensely active in the normal happenings in a cell. Pentoses are combined with phosphate and special nitrogen-containing molecules to form a group of substances found in all cell nuclei, the nucleins or nucleic acids; and, strangely enough, one pentose is very generally present in all animal nuclei, a different one in those of plants. A pentose, again combined in part with phosphate, in part with a different nitrogen-containing molecule, is present in one large group of fatty substances, as we shall see; and still others, partially combined with proteins and other special molecules, play a widespread and important role in cells as catalysts.

Fats

The fats or, more generally speaking, the lipins (fatlike substances) are in several senses a three-ring circus. First of all, there are three major types of substances, some really not at all related, which are called lipins because all dissolve in the same

sort of solvents, as ether or benzene. Second, in the actual structure of individual fat molecules, the same unit is repeated three times. And finally, even in a completely technical sense, the molecules in one group, the sterols, are composed of atoms actually grouped into a number of closed rings.

What a merry chase they have led scientists! The carbohydrates, except for those with the largest molecules, are readily soluble in water, and when the water is evaporated they tend to separate out as nice pure crystals. Even with a solution of a mixture of different sugars, like molecules come together when they crystallize, so that the different substances separate into distinct solid masses, though these several kinds of crystals are
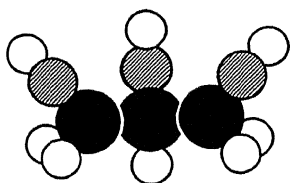
FIG. 27. A molecule of glycerine.

roughly mixed together. Not so the fats. While not dissolving in water, the whole pack and parcel of them will dissolve in hot ether, and when this is cooled or evaporated they form either no crystals at all or an unsatisfactory type of so-called liquid crystal and, what is worse, one substance remains dissolved in another even when this is solidified. It has, therefore, taken enormous labor and ingenuity to get these separate substances apart for study in a pure form. Even today in the more complex members of this group, many mixtures are still unresolved. Fortunately, for many purposes it makes little difference whether or not the absolutely pure fats are separated out; large groups are so nearly identical in their composition and behavior that they can be studied en masse.

The true fats, the first great group of lipins, are both simpler and more complex than the carbohydrates. A fat molecule is formed by the combination of one molecule of glycerine

$$H \quad H \quad H$$
$$H-\overset{\displaystyle |}{C}- -\overset{\displaystyle |}{C}-\overset{\displaystyle |}{C}-H$$
$$\overset{\displaystyle |}{O} \quad \overset{\displaystyle |}{O} \quad \overset{\displaystyle |}{O}$$
$$H \quad H \quad H$$
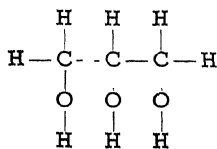
(which misses being a triose only because it has no oxygen jaws at one end) with three molecules of fatty acid. It is therefore more complex than a mono- or disaccharide; but there it ends, for these molecules are not further knotted to one another to form still larger ones. A fatty acid is something like a match stick, or a freight train, as fancy may see it.
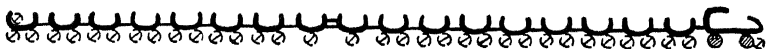
FIG. 28. A fatty acid molecule is something like a freight train.
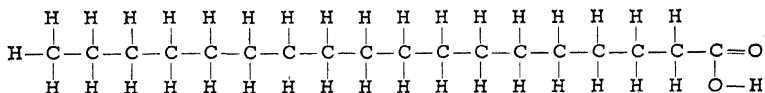
At one end, where the two oxygen atoms are located, there is a certain active individuality; in fact, this carboxyl group, as
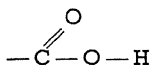
$$-\overset{\displaystyle O}{\underset{}{C}}-O-H$$ is called, is what gives to it its acid properties

and is the one that combines with the —OH in glycerine. But the rest of its long length is as drearily similar as a train of box cars. The most common fatty acids have 16 or 18 CH_2 units hung on one behind the other, though ones with other numbers are not rare. Others acquire a certain added individuality, and for that matter a greater chemical activity, by having here and there along their length two of the carbon atoms doubly attached to each other, with the loss of some of the combined hydrogen; much as an unbroken line of box cars is relieved by an occasional flat one. A single fat molecule may have three identical or three different fatty acids combined to
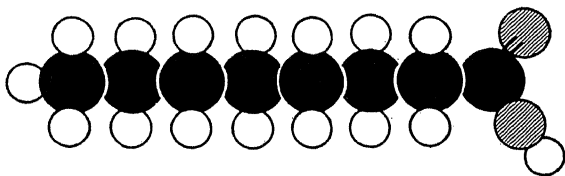
FIG. 29. A molecule of a very short fatty acid (a common one in butter).

its glycerine, so obviously a great variety of simple fats can exist; and do, all mixed up together.

On standing outside of the body, fats become rancid, in part at least because the glycerine combination is broken and free fatty acids appear. This same decomposition is produced rapidly by boiling with lye, but instead of the fatty acids their sodium or potassium salts are formed. These are our common soaps.

The fatty acids escape from utter desuetude only by the carboxyl group at their end. When the oxygens are missing and there remains simply a chain of carbon atoms with a fuzz of hydrogen atoms along its length, the molecules become members of the great inert group of hydrocarbons, ranging from marsh gas (methane) through kerosene and petroleum to ordinary paraffin wax. It is probable, in fact, that just such a gradual loss of oxygen through geological epochs, under the conditions of heat and pressure within the earth, has changed the fatty acids of huge numbers of dead organisms into natural oil and gas.

Under ordinary conditions these molecules are extremely stable and chemically inactive, which is the reason they keep so well and are used, as vaseline or paraffin, to coat objects and so prevent their changing. But one powerful and important reaction these hydrocarbons do show. Ordinarily they will not combine at all with oxygen, but if they are sufficiently heated, or if catalysts are present, then they oxidize rapidly with the liberation of large amounts of energy, and may actively burn or even explode.

Since carbon atoms are always potentially able to combine with two atoms of oxygen, all organic compounds are combustible and can be "burned" to the fully oxidized form, carbon dioxide. Sugars, fats, proteins, creatine, lactic acid, and all the rest, if catalyzed in the presence of oxygen, end up as carbon dioxide and water and perhaps some nitrogen derivatives. Since fat molecules have the least oxygen in proportion to carbon to

begin with, they obviously can take on more oxygen and therefore give off more energy than any other organic compounds except the hydrocarbons. Weight for weight, fats give more energy on burning than the other body substances, and it is not surprising, therefore, to find them a favorite means for storing reserve energy. Pure hydrocarbons would hardly work, since their great chemical stability and practically complete insolubility in watery protoplasm would make them very unmanageable. The fats, being more like soap, become spread about in water in a semi-dispersed fashion and, since the molecule has an active end, they are also more easily utilized by cells. Even so, the oxidation of fat seems to present a certain amount of difficulty, for sugars, which are already partially oxidized as compared to fats, are almost always preferred by cells as their immediate fuel.

Carbohydrates, then, represent the fluid reserve of cells against the depression of food lack, whereas fats are the great bulk of frozen assets. In many animals masses of special cells are set aside as tiny reservoirs of fat, and these cells come to be composed of a great droplet of this material surrounded by the thinnest possible rim of active protoplasm. Incidentally, it is the proper distribution of masses of such fat cells beneath the skin which, in judicious amounts, gives the delightful contours to human anatomy. But a fat man, with great amounts of stored fat, will feel hungry and exhausted at least as quickly as a thin one after a given time since eating and with the same amount of exercise carried on in the interval. It is the carbohydrate store which serves these immediate needs from hour to hour, and this is promptly replenished through the food. Over a longer time, however, it is the fat that counts; and in facing starvation, as most living things have to do often enough under natural circumstances, the fat individual will long survive his skinny confreres.

Man burns almost identical material, therefore, except for coal, in his machines and in his body. Gasoline, a hydro-

carbon, is carefully mixed with oxygen and exploded under pressure, and the energy produced by its burning drives our cars. The very similar fatty acid of fat molecules is similarly burned, though in a far more elegant and efficient manner, to drive our muscles.

The second group of substances in the great class of lipins, the phosphorus-containing fats, or phospholipins, are closely related to the true fats. The difference, in fact, lies merely in this: instead of being combined with three fatty acids, the glycerine in the phospholipin molecule is combined with only two and the third position is occupied by a complex of phosphate and nitrogenous substance.

$$
\begin{array}{c}
\text{H} \\
| \\
\text{H} - \text{C} - \text{O} - \text{Fatty acid} \\
| \\
\text{H} - \text{C} - \text{O} - \text{Fatty acid} \\
| \\
\text{H} - \text{C} - \text{O} - \text{Phosphate-choline} \\
| \\
\text{H}
\end{array}
$$
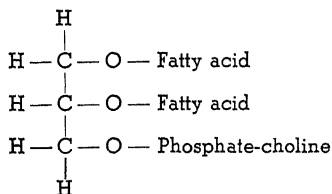
Because of these extra groups, the phospholipins (of which lecithin, composing much of the yolk of egg, is the most important) are chemically somewhat more alert than the true fats and are also distinctly more soluble in water. A drop of lecithin placed in water under proper conditions behaves like a chicken in the road, or any other animate system that doesn't know its own mind. A large part of the molecule, being fatty, is quite insoluble in water; the remainder, with phosphate and a type of salt, is very soluble. Some sort of internal tussle seems to occur within individual molecules or masses of them. The lecithin starts to dissolve, becomes spread out in long threads through the water, then withdraws again, so that a gently writhing tangle of lecithin in water and water in lecithin is the result. This improved solubility has the most important consequences; for, as we shall see in the next chapter, phospholipins constitute an integral part of all protoplasm. The true fats are less soluble and, like the polysaccharides, are never quite accepted into this

molecular society, but exist within the cell as separate droplets or masses of clannish molecules.

The last great group of lipins, the sterols and their terpene relatives, are to a large extent no true group at all but simply the et cetera of the classification. There are included, to be sure, many kinds of closely related compounds, but some of the types of substances are chemically rather far from others. All are composed to a large extent of carbon and hydrogen, with a few atoms of oxygen tucked here and there in the molecule. The atoms are not arranged in simple chains of beads, but make closed loops with cross bridges of a bewildering variety. Many have a "skeleton" of three (or four) rings, but the arrangement of these rings and the number and even kinds of atoms that compose or are attached to them show great variation.

This category, though known for a long time (various sterols and waxes were among the early substances isolated from bodies), has only in recent years leaped into great prominence.
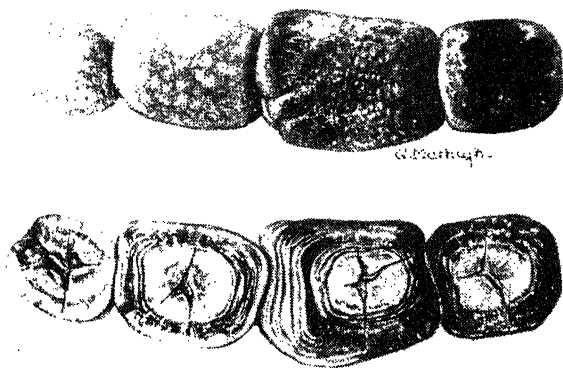
FIG. 30. Four gallstones from one gall bladder. Above, whole; below, in section. Note rings of deposited cholesterol, pigment, etc. (Photograph by courtesy of Dr. D. B. Phemister.)

Cholesterol, the best known and by far the most plentiful and widespread of the group, was early discovered in gallstones (the name means the solid in bile). It is now recognized as a universal constituent of protoplasm, like lecithin, and the two are often most intimately related in cell structures and in their effects on cell behavior.
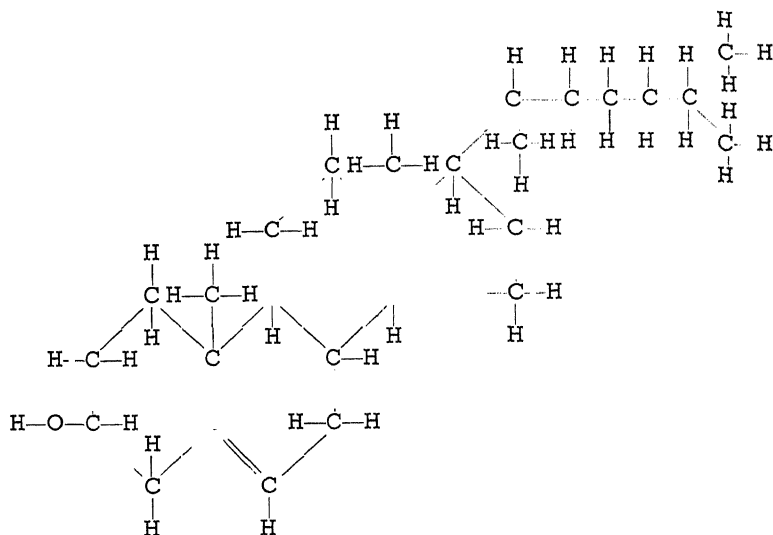
Cholesterol

Many new substances, some essential to health or life itself, some discovered only in recent years, others known for a longer time but only recently identified, have been shown to belong in this group.

The vitamin that prevents rickets, calciferol, is almost, but not quite, identical with cholesterol. The male sex hormone required for the full development of masculine structures and function, as well as a female sex hormone essential to the analogous changes of pregnancy, are likewise but slightly modified cholesterol molecules; interestingly enough, changed by oxidation or reduction in opposite directions relative to their prototype. Other closely related substances have been

shown to be effective in causing cancer and may normally be concerned in some still mysterious way with the whole question of growth. Another group has been definitely shown to control growth in plants, and still others form portions of vitally important enzyme molecules in cells, or of the bile acids, important in digestion. The digitalis drugs, so valuable in treatment of a failing heart, also are sterol compounds. Truly, this is an enterprising family, the sterols, for, close chemical relatives that they are, its members have found a rich variety of independent biological occupations.

Proteins

The proteins, universally present in living matter, aristocratic yet plebeian, infinitely diverse and individual yet all built from the same twenty-odd amino acids, remain the most important and most tantalizing of substances. An amino acid has two distinctive chemical groups, a carboxyl group like that of the fatty acid, and on the carbon next to it an amino group, NH_2.

$$H - N - C - C - O - H$$
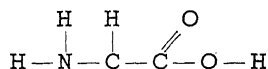
(R is a simple or complex collection of atoms)

Just as the carboxyl group is acid, so the amino group is basic, and the opposite groups of separate amino acids can therefore easily combine, first in the form of salts and then with the loss of water (as, indeed, occurs when two glucose molecules combine or a fatty acid and glycerine) into a more permanent and stable combination.
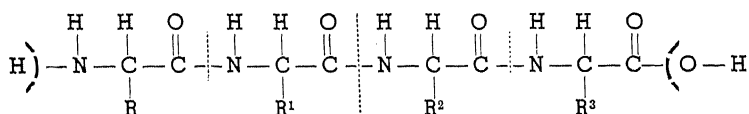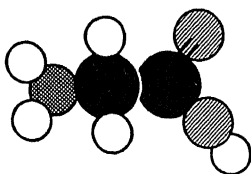
FIG. 31. A molecule of the simplest amino acid (glycine).

When enough of these units are thus hooked together, a protein molecule is formed.

All amino acid molecules are alike in structure so far, and each is different beyond. The simplest of all, glycine, has no more to the molecule, just an extra hydrogen to round it off

$$H-N-C-C-O-H$$
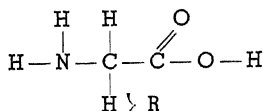
and the most complex, tryptophane, has a whole dog attached to this tail, containing two complete rings of atoms.
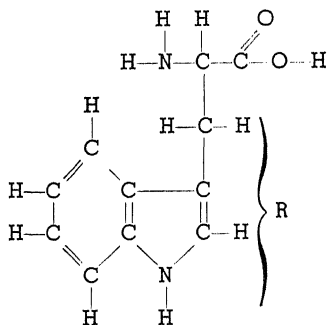
Several of the amino acids contain sulphur atoms in the molecule, many contain additional oxygen or nitrogen atoms or both. Short chains of particular amino acids, di-, tri-, or polypeptides (like the di- and polysaccharides) exist as separate molecules in cells and often have important duties. The tripeptide, glutathione, for example, plays a vital role in cell respiration. Certain individual amino acids, including tryptophane, are essential to the life of the mammalian body yet cannot be made by its cells; for if any one of them be absent from the food, death ultimately results. The thyroid hormone is formed by

adding atoms of iodine to a doubled amino acid, and another hormone, adrenaline, by a slightly different modification of the same amino acid, tyrosine.

The chemistry of the amino acids (at least all those so far discovered—and two new ones have been found within the last five years) is well understood, and even short polypeptide chains have been built. But biologists are only beginning to explore seriously the role and importance of these smaller molecules in the normal activity of cells because of the overwhelming importance of the greater groups, the proteins. As starch or glycogen is built from large numbers of glucose molecules, so the proteins are formed by stable combinations of amino acids, not in dozens or scores but in hundreds or thousands. A molecule of albumin, the main component of egg white and one of the simple proteins, contains nearly 300 individual amino acid molecules; and one of the more complex proteins present in the blood of the king crab contains 100 times as many. But it is not just because proteins are built of more units than polysaccharides that they are so vastly more important—six-syllable words are not necessarily better than those of two.

Carbohydrates are words repeating one letter, whereas protein words contain the whole alphabet. Starch, glycogen, dextrose, cellulose, and all the other large polysaccharides are built solely of glucose molecules in more or less the same mold. But proteins, with varying numbers of the twenty-three or more different unit amino acids, arranged in any order, present in widely different proportions, and possessing marked individual peculiarities, have as rich an individuality as the entirety of the English language. It is again the story of 159 and 915, of "rat" and "art"—with different kinds of units, pattern of combination, far more than simple reduplication, throws open the wealth of permutations. It is not difficult to calculate how many possible arrangements of ten letters could be made, and the answer is over thirty million; when twenty-three different letters are

taken in groups of several hundred, the possible number is
legion. Clearly, if each particular combination of amino acids
gives a unique protein, an unlimited number of these can exist.
And, as certain letter sequences have come to be meaningful
words, so certain combinations of amino acids have come to be
actually existent proteins. Also, as in words, there will be
greater and lesser degrees of similarity between individuals.
It is hardly surprising that the proteins have presented scientists
with such a tremendous crossword puzzle.

We often speak of "protoplasm" as we do of "man" as if it
were a real thing. Both, of course, are abstractions. What does
exist is the protoplasm of any particular cell, though even this
would be hard to define accurately. It is true, however, that
the protoplasm of all cells of one type, say in the livers of all
men, is the same—at least by any measures we can yet apply
to it. The protoplasm of a man's muscle cells, however, is dif-
ferent from that of his liver cells, and new again is the proto-
plasm of a dog's liver cells. There are, then, as many kinds of
protoplasm as there are kinds of cells in tissues and species,
many millions at least. But this is still loose talk, for no one
knows exactly what protoplasm is, let alone how one proto-
plasm differs from another. What is clear and can be shown
convincingly is that some of the proteins present in the proto-
plasm of any one kind of cell are unique to it. Even more: the
more alike two cells are, the more closely alike are the pro-
teins they contain; the more widely different the cells, so also
their proteins, or at least some of them.

Several intriguing facets of science have to do with this
specificity of proteins, but this intellectual dish must be re-
served for another meal. Suffice it for the present that proteins,
many of them, are present in every living cell and organism.
With their nitrogen, assortment of amino acids, huge size, and
great complexity, they are the keystone in the chemical arch
of life. They are built intimately into the architecture of cells,
contribute greatly to the colloidal structure of protoplasm, and

are an integral part or an essential carrier of most, if not all, the enzymes which govern the flux of metabolism. How their crucially important physical and chemical properties emerge from the multiple combination of amino acids, which are after all fairly simple molecules, is still but dimly, though with ever-increasing clarity, understood. This much seems certain: no sharp break occurs in the governing laws of matter, and the nature of proteins is just as completely dependent on their constituent atoms and groupings as are the properties of water on its. Whether, in either case, a complete analysis in terms of constituent units is attainable is at present debated in the realm where science and philosophy meet, but in neither case is there reason to suppose that some novel, mysterious, and vital influence is at play.
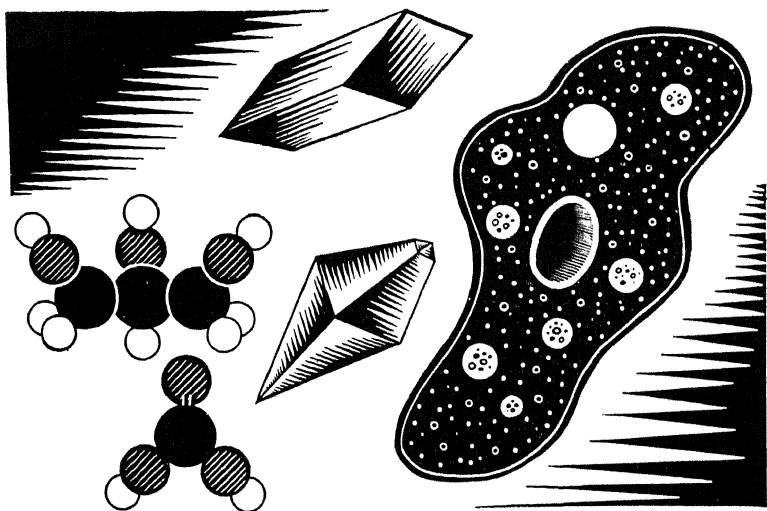
Chapter Four: Architecture in Miniature—
Protoplasmic Structure

The molecules found in protoplasm range from those of simple water and salt to the complex ones of carbohydrates, lipins and, especially, proteins, which are probably the most elaborately organized atomic groupings that exist in the universe. And the arrangement of these molecules in protoplasm likewise shows the most intricate organization. How bandied about is the word "organization"! We "organize" our lives, "organize" a society, "organize" a whole state, until its implications become blurred and cheapened. Yet organization remains one of the great riches of the universe, the most important increment in value.

The primary generalization of physical science, dignified by the resounding phrase, "The First Law of Thermodynamics," states that: the total amount of energy, or more accurately of matter and energy, in the universe or any completely isolated system remains unchanged, whatever whirl of events occurs within it. Of course this generalization is not proven for the

whole universe, but the careful observations and brilliant analysis of Lavoisier, before he was swept away in the flood of revolution in France, and of Helmholtz, quietly working in nineteenth-century Germany when that stricken country did not export brains so lightly, have been only refined and substantiated in succeeding decades, through the final brushing aside of the boundary between matter and energy in our own time by Einstein. It is a matter of empirical observation that, on every test and to the finest accuracy of which scientific investigation is capable, this law has held.

But we are more interested now in the second great generalization, for the Second Law of Thermodynamics states that energy is becoming less available, that power reserves are running down, in short that the universe is becoming ever more disorganized, more chaotic. Here again all terrestrial observations announce its accuracy, yet extrapolations to infinity in space and in time seem more dubious. The intellect hesitates at the conception of a unidirectional universe with a wound-up beginning and a run-down end, despite our intuitive recognition of time's arrow; and many scientists consider it possible that this degradation may be reversed elsewhere or at other times. Be this as it may, it is no less important that the universe be organized than that it exist at all, for in chaos time stands still and life could not happen.

A swarm of molecules beating hither and yon in the air of a breathless summer day are in a state of chaos. Their movement is random, their collision is accidental and perfectly symmetrical and, though the molecules may dart about at the speed of a quarter of a mile per second, they get nowhere; for should one chance to be jostled to a new position in the swarm another will promptly replace it. Energy and matter are here, but unorganized. Nothing happens or can happen except as a change occurring outside of this system acts upon it. Yet such a mass of molecules, unchanged in amount of matter or energy, but with organization increased, could become a terrific force.

If all were to move simultaneously in the same direction, which means, of course, organization, what a wind it would be! Jostling molecules would never spontaneously become uniformly oriented, but the reverse change is the regular occurrence noted in the "Second Law." When the motion of wind or any other form of energy is "expended," it reappears ultimately as heat, and heat is unorganized molecular movement.

Man may claim with pride a place in the stream of life, for living things have the most highly organized systems that exist. Their molecules are not all mixed at random, jostling their accidental ways through protoplasm, any more than the stars scatter through the skies by chance. Throughout, individual units are grouped in non-random manner, are integrated into more elaborate units, and so through hierarchy upon hierarchy. In the sidereal universe, a system of planets and sun is but one unit in a disc-like galaxy and these are points of matter in the still more enormous supergalaxy and so on beyond human knowledge. Turning inward we find our bodies comparably composed of many organs, each with several tissues built from many cells; and within the cell, no less, as we throw upon it ever higher powers of our microscope, are droplets and granules, mitochondria and Golgi apparatus, membranes, nuclei, and chromosomes. When examined beyond the range of microscopic vision, the remaining "fluid" protoplasm again loses its homogeneity and is found to be built up from particles and aggregates with as much structure and complexity as a whole starry galaxy.
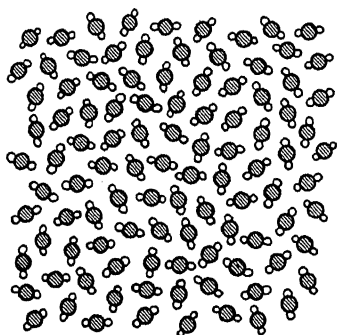
Fig. 32. Molecules of water slip past each other in liquid water and are located at random.

A given mass of molecules, say of water, has far less organi-

zation as a gas than as a liquid, for in the liquid the mole-
cules are close enough together so that some of the electric
pull which normally holds atoms together within the molecule
is felt from one molecule to another. Partially held by these
forces, the molecules tend to enter into patterns; and liquid
water is no longer, as is water vapor, composed simply of single
water molecules, but rather of combinations of them. Two,
three, or more single water molecules combine (polymerize)
to form larger ones, $(H_2O)_2$, $(H_2O)_3$, etc. These attractive forces
continually act to make the molecules settle together into the
most compact arrangement possible, but the ceaseless molec-
ular movement prevents this from succeeding too well.

When the thermal movements are violent enough, that is,
when a liquid is warmed sufficiently, the attraction is overcome
and the molecules fly apart to form a gas. When the move-
ments become less, because of cooling, then the attractive
forces prevail, the molecules are pulled into place in a regular
pattern, and the liquid freezes. Layer on layer, the molecules
settle in equivalent positions, until there results a solid mass of
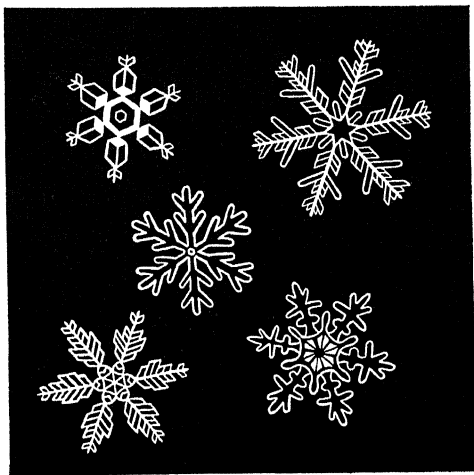
FIG. 33.   Snow crystals.   When water freezes, the molecules form
regular patterns.
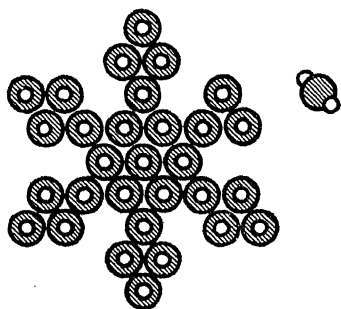
FIG. 34. Water molecules gathering in patterns to form a crystal. Each molecule has small hydrogen atoms on either side of a large oxygen atom. A molecule is just entering the crystal.

definite repeated structure, a crystal. Sit in the window at the next snow storm and watch the variegated water crystals before they melt on the glass. Of myriad sizes and structural detail, every one still shows the same fundamental pattern, the six-pointed star. This is as truly the expression of some essential character of water molecules as are the five-pointed stars of our flag a symbol of the American people.

So much for a group of uniform molecules, but what of the situation when many different kinds are mixed together? It is hardly strange that molecules of some one particular shape and with like surrounding forces should more easily congregate together than with those which are different. There is a strong molecular clannishness; and when a mixture, say of water and alcohol, is progressively cooled, there form separate crystals of ice and of solid alcohol, each containing molecules of its own kind to the exclusion of the other. Like attracts like in molecules far more certainly than in human beings, but there is a limit to these castes and miscegenation does occur. When many different kinds of molecules which are very similar chemically, as in the case of the different fats, are present together; or when certain molecules are present in small numbers in a mass of some other kind; and, especially,

FIG. 35. When molecules of many kinds and sizes are mixed together, the solid formed is not a regular crystal but a confused amorphous mass.

when very large and cumbersome molecules are mixed with small ones, then the social lines are broken and a disorganized amorphous solid mass results.

Protoplasm is neither liquid nor solid, neither crystalline nor amorphous. Many of its substances are dissolved in water, others are frankly excluded and many more are neither in nor out. The water in cells has dissolved in it the great variety of simple soluble substances—salts, extractives, simpler sugars, and other individual molecules — which jostle about among the far more numerous water molecules. This is the situation in any ordinary watery solution. At the other extreme, large clumps of quite insoluble molecules, forming microscopically
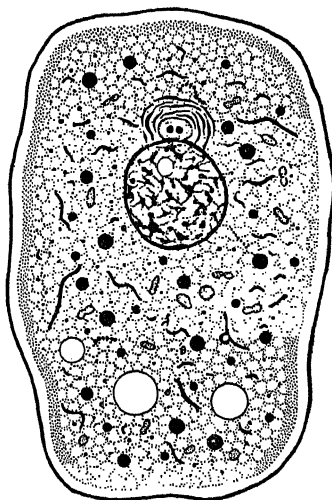
FIG. 36. A typical cell, showing microscopically visible structures.
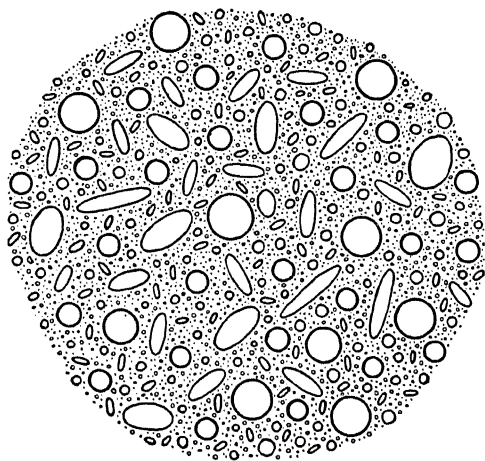
FIG. 37. Ultramicroscopic structure of a bit of the homogeneous protoplasm occupying the clear spaces between the structures visible in the above figure.

visible granules of glycogen or drops of fat, are separate islands
in the salty sea. In between are the colloidal lipins and pro-
teins. On these tiny particles, precariously suspended in their
watery world, tremble dangerously yet surely many of the most
characteristic attributes of life. Some of these particles are really
single giant molecules, as in the case of the proteins, others are
small bands of like individuals presenting a solid front to their
strange surroundings and yet, while not dispersing, also never
joining their fellow groups.

Counting Atoms

Before improving our acquaintance with the colloids we must
find out how it is possible to talk so positively of the shape and
size and distribution of particles far beyond the range of vision,
even as extended with the tools of science. Obviously, if the
weights of atoms are known, these can be added to get the
weights of the molecules built from them. The atomic weights,
relative to that of the hydrogen atom, are obtained by accurately
weighing the amount of one pure substance which will just
completely react with a standard amount of another. Only
when equal numbers of molecules of both substances are pres-
ent can all be completely changed, without some of one kind
or the other being left over; so that the relative weights of the
substances must also be the relative weights of their molecules.
By comparing enough of these molecular weights and noting
the atoms present in each molecule, a set of atomic weights can
be obtained. For example: Two grams of hydrogen gas (H_2)
combines with 70 grams of chlorine gas (Cl_2) to give 72 grams
of hydrochloric acid (HCl). No atoms are made or lost in the
change of molecules so the reaction must be:

$$H_2 + Cl_2 \longrightarrow 2\ HCl.$$
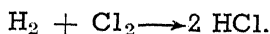
Each atom of chlorine, therefore, weighs thirty-five times as
much as one of hydrogen.

A large number of such determinations, laboriously carried
through during the last century, has established the important

table of relative atomic weights. If, then, we could only determine the number of molecules in a known weight of any particular substance, these relative atomic and molecular weights could at once be placed on an absolute basis. One gram of hydrogen atoms, actually two grams of hydrogen molecules (H_2) since it is impossible to deal with single atoms, is taken as a unit amount of matter and called one mole. (More precisely, with oxygen as standard, the hydrogen unit is 1.008 grams.) In the form of a gas and under correct conditions of pressure and temperature, this much hydrogen occupies a volume of 22.4 liters or about one cubic foot. Whatever the actual number of hydrogen molecules in this mole, the same number of molecules must be present, by definition, in a mole of any other substance; for a mole is its molecular weight expressed in grams. The oxygen atom, for example, is sixteen times as heavy as that of hydrogen, the water molecule is H_2O, so a molecule of water weighs eighteen times as much as a hydrogen atom. In a mole of hydrogen are enough hydrogen atoms

FIG. 38. The molecules in a teaspoonful of water would become a gallon of water vapor.

to weigh one gram and the same number of water molecules in a mole of water must weigh eighteen grams, about a tablespoonful. Yet, when vaporized, this small amount of liquid water comes to occupy exactly the same large volume as does a mole of hydrogen molecules. In the gaseous state, equal numbers of molecules occupy equal volumes.

No wonder that when water boils enormous pressures are developed; no wonder that when the molecules of solid gun-

powder are suddenly changed chemically into an equivalent number of small molecules in gaseous form a terrific explosion results. The space occupied by a mole of water molecules as a gas is about twelve hundred times as great as that occupied by them as a liquid, so that if the molecules are packed together in water, they must be separated by relatively enormous distances in water vapor. Less than one-thousandth of the space "filled" by the gas is occupied by molecules. The rest is empty. Each molecule dashes about in an average volume which depends rather on its speed than on its actual size. The hotter the gas, the faster its molecules move and the more space each tries to occupy, as evidenced by an increase in the volume of the gas or an increase in the expanding pressure if the volume is held constant. At any one temperature, all gas molecules move with the same energy and for this reason equal numbers occupy the same volume despite differences in the actual size. In the same way, the number of hawks over a square mile of woodland is not related to the size of the birds but rather to the range each one can cover and defend as its own private hunting ground.

Surely, then, there are not really many molecules present in a cubic foot of gas. A million? But remember that molecules must be extremely small. Shall we guess safely, a million times a million? Way off! A million times as many is not enough, for the number of molecules in a mole is 6.064×10^{23}, over six times a million times a million times a million times a million! How dare scientists be so bold as to claim that just this, and not .01 of one per cent more or less, is the correct number when no molecule has ever been seen? No, not seen but none the less counted.

It has been possible to take a picture of the path of a single ion, or better of a single electron split from an atom, because it disturbs other atoms which it passes and can produce a minute thunder shower in a water-saturated gas. When radium or other radioactive substances decompose, individual atoms explode

and shoot off rapidly moving electrons and other particles which, by virtue of their terrific speed, excite these thunder storms or stimulate luminescent molecules to emit light. If sufficiently few radium molecules are present, the explosion of each one is recorded by a tiny flash as projectiles from it penetrate the surrounding swarm of luminescent molecules. A mixture of a tiny bit of radium or one of its relatives with a large amount of the luminescent substance (zinc sulphide) constitutes the material which is painted on the hands and numbers of radio-lite watches to make them visible in the

FIG. 39. Paths of alpha particles shot out from a source just below the center of the photograph. Note a collision on the left. (Photograph by courtesy of W. D. Harkins.)

dark. With an ordinarily good hand lens held over the watch hand in a dark room, you can readily see the flash of single exploding atoms. This is an adventure to be tried; no sky on a twinkling starry night appears so beautiful!

FIG. 40. A luminous figure on a watch dial looks something like this, through a hand lens, in the dark.

Well, then, it should be possible, is indeed relatively easy, to count the total number of atoms exploding per second when a certain small weight of radium is present. We also know, by measuring more grossly the total radioactive power of a lump of radium, that exactly half the atoms present will have been shattered in fourteen hundred years. The rest, of course, is simple enough, for one measure-

ment gives the actual number of atoms breaking down in a certain time and the other tells what fraction of all the atoms present would break down in that time. Multiplying the two figures together gives the above stated number.

One such means of measuring the number of molecules might well have unexpected sources of error, and it is very reassuring to know that at least six different methods—as unrelated as is the breakdown of radium to the spreading of oil films on water or to the amount of silver which can be electroplated by a given amount of electric current—permit the calculation of this number, and all agree surprisingly well as to its exact value. One hydrogen atom, then, weighs $\frac{1}{6 \times 10^{23}}$ gram, and, if a sphere, would be approximately $\frac{1}{10^8}$ centimeter in diameter. Even an enormous protein molecule, three million times as heavy as hydrogen, would weigh only $\frac{1}{2 \times 10^{17}}$ gram, and would be only about one hundred times as great across, or one-millionth of a centimeter.

The human eye can distinguish, under the best conditions, two points separated by about one-hundredth of a centimeter. Good microscopes magnify objects two to three thousand times, but at the same time produce some blurring, so that with their aid particles of about 1/10,000 centimeter, possibly even down to 1/100,000, might barely be distinguished as such. If the individual particles are made to shine in reflected light, however, as the tiny motes of dust floating in the air shine when they enter a beam of sunlight and are looked at from the side; when, in other words, an ultramicroscope is used, tiny shiny dots of considerably smaller size can be distinguished.

Perhaps we have actually seen individual protein molecules. Perhaps the tiny particles visible in protein solutions under the ultramicroscope are still small clumps of these molecules. The former is not unlikely. We have reached, then, a junction between the smallest things we see and the largest we infer, just

OBJECT	SIZE	
	Micra	
Amoeba	100	
Sea Urchin Egg	70	
Liver Cell	20	
Red Blood Cell	7	
Prodigiosus Bacillus	0.7	

	(Size magnified 100 times) Millimicra	
Prodigiosus Bacillus	700	
Typhoid Bacillus	250	
Rabies Virus	125	
Tobacco Mosaic Virus	40	
Gene	35	
Hemoglobin Molecule	7	

	(Magnified another 100 times) Millimicra	
Hemoglobin Molecule	7	
Egg Albumin Molecule	4	
Amino Acid Molecule	0.7	
Salt Molecule	0.4	

FIG. 41. A scale of sizes of some objects, from salt molecules to cells.
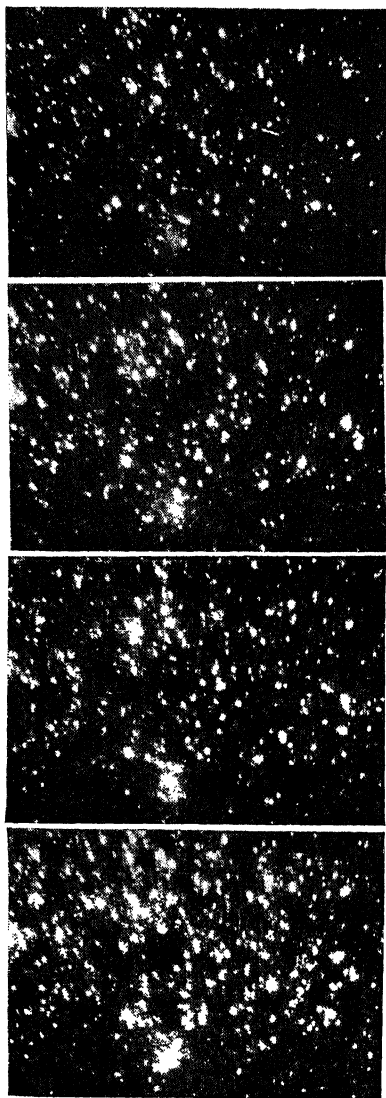
as when two borings started at opposite sides of a mountain join accurately to form a through tunnel. The two domains will soon overlap freely; for the new "electron microscope" promises to make visibility leap forward to tenfold smaller objects, while at the other end, protein molecules ten times heavier than those previously known have been newly discovered.

Like Glue

The white or albumen of a raw egg is a fairly concentrated source of a few proteins. It is not difficult to purify the most important of these, called egg albumen, and even to obtain it in large regular crystals. Such crystals, shaken with water, will slowly dissolve until no solid is left, and none will settle out again though it be allowed to stand indefinitely. The protein has gone into solution. Yet somehow things don't seem right for, unless

FIG. 42. Four frames from a moving picture of a colloidal suspension. The single particles are luminous by reflected light in the ultramicroscope. The changes in position are due to Brownian movement of the particles; the large ones remaining at the left-hand edge and the lower center of each photograph show that the same solution is in the microscope field. (Film by courtesy of H. B. Lemon.)

we have added very large amounts of water, the resulting liquid is not clear, limpid, and completely fluid, as are even concentrated solutions of most solids, but rather a little thick or viscid, opalescent, and, in fact, of a somewhat gluey disposition. The word "colloid" means gluelike, and was given to this kind of a solution because Graham, who christened it, made his observations on glue. The large single protein molecules, or aggregations of a few of them, are so disproportionately huge compared to the tiny water molecules in which they float that the effect is that of a minute solid grain suspended in water.

The really important thing about such particles is that they present a surface of contact to the surrounding molecules. It is sometimes difficult to see why there is no "surface" between two or many roughly similar molecules mixed together, while there is a perfectly good one between groups of different kinds of molecules massed according to species but in contact with each other, as between oil and water. Yet it is the same principle that enabled a handful of soldiers to face a horde of at-
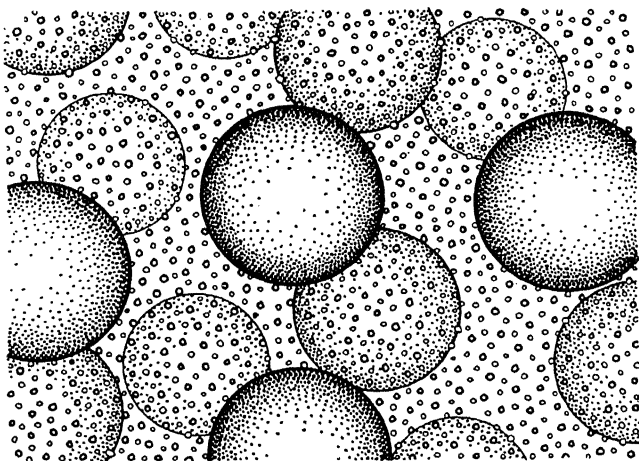
Fig. 43.   The colloidal particles are huge compared to the water molecules in which they float.

tacking Indians. With white and red men all intermingled, the situation and its results were quite different from what they were when the whites formed a hollow circle or square and presented a continuous front to their opponents. In the latter case a surface was present.

The most important and unique properties of colloids derive from the truly enormous surfaces present in a small amount of solution. As a solid mass is fractured, two new surfaces are formed for each split, though the total mass of solid is unchanged. As it is progressively crushed and ground and pulverized, the only real change is an increase in surface; yet how different are the properties of a steel ball and of steel powder, which, when exposed to air, burns up like dry pine needles. But the finest grain of dust prepared mechanically is still a population of billions of molecules. It is easily visible under the microscope and, if insoluble in water, will settle out from the liquid quite rapidly. It is not surprising, then, that, when
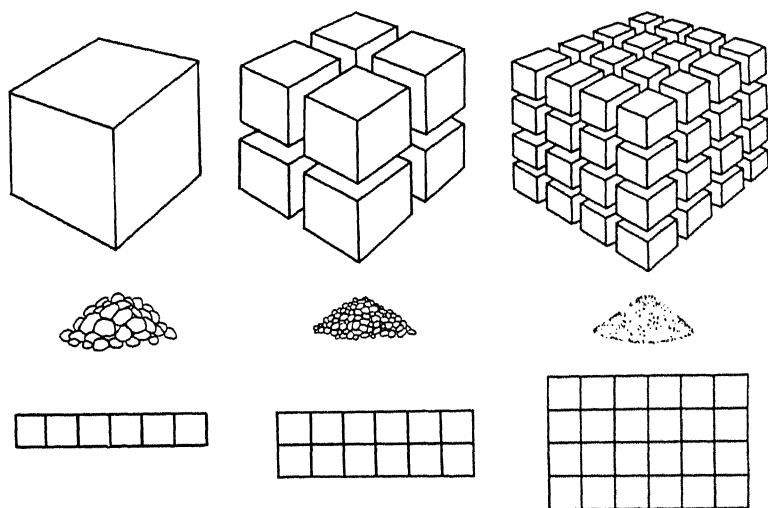
FIG. 44. As a block of material is broken into smaller bits—say from stones to pebbles to sand—the surface for a given mass increases as shown by the squares.

such a grain is still further divided thousands of times and its surface correspondingly increased, the change in properties is so great as to yield an entirely new state of matter.

Such, then, is a colloidal solution, composed of myriad tiny bits of matter floating in a great ocean of an alien kind. If concentrated, the colloidal particles (the dispersed phase) may be in close contact and occupy a large portion of the total volume, though surrounded by molecules of water (the continuous phase). If dilute, the individual giants may blunder their way among enormous numbers of water molecules in magnificent isolation from their fellows, only occasionally coming within one another's range of influence. In fact, such a solution is not dissimilar to our heavenly universe, where also mammoth particles pursue their lonely ways through space.

In another manner, too, the colloidal solution suggests the heavens. If looked at under the ultramicroscope, not only do countless tiny stars mark the presence of the particles, but these stars dance and twinkle. Seen first by Brown, when he floated some minute lycopodium spores on water, this dancing is known as Brownian movement. No particle is still, each jerks about erratically, making tiny dashes hither and yon only perpetually to bounce back again. It looks perhaps like a milling crowd or a crowded dance floor seen from high above, or like a swarm of darting midges; and it is indeed a manifestation of, and the most direct evidence for, the deathless dance of molecules.

Individual molecules careening along their headlong paths collide, ricochet, and impart their energy to one another. A large molecule moving relatively slowly would bat out a small one, if hit squarely, at a tremendous speed. A small one hitting a large might only jar it, like a marble thrown at a bowling ball. But a large molecule, and still more a colloidal particle, offers a target for a great many small ones, so that it is being continuously bombarded from all directions. These many random blows tend to average out and cancel their effects in all
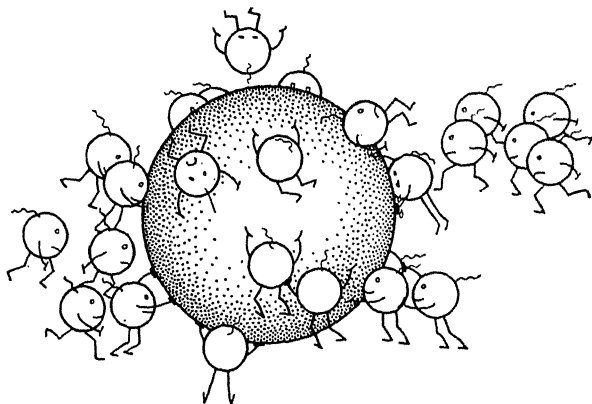
FIG. 45. Water molecules play an unending game of pushball with tho largo
colloidal particles.

directions and so leave the large hulk unmoved. When, by
chance, however, the barrage from one direction becomes
hotter the particle is driven forward until, again by chance, a
counterattack starts it in a new direction. As a huge push-ball
is driven erratically about by the shoves of many hands, so a
colloidal particle is driven on its irregular path. The larger the
particle, the greater the number of molecules hitting it from all
directions, the less the chance of an accidental overpowering
from any one side and, with its greater inertia, the less its move-
ment. It is possible even, by making continuous records of the
movements of a particle, to calcu-
late from its speed and distance
of motion its size as compared to
that of the bombarding molecules.
Again, the figures reached agree
closely with those obtained from
other evidence.
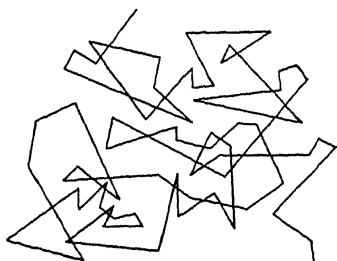
FIG. 46. Actual path of a colloi-
dal particle in Brownian move-
ment.

Now why should particles, no
matter how small, remain sus-
pended in water? If heavier, they
should fall like sand grains; if

lighter, they should rise like oil droplets. Certainly, except in the unusual case of their having exactly the same weight, or more accurately the same density, as the continuous liquid, such dispersed particles should separate out. The conditions that keep them afloat have to do largely with the enormous surfaces—which we shall examine later—but one factor is their perpetual movement. Gravity steadily pulls all particles down, but the endless jostling by those below just as continuously bounces up the ones above them. An equilibrium is reached, with more particles in the lower layers of the liquid and fewer in the upper, exactly as occurs with air. Even with the single gas molecules, there is much closer packing, greater air density, at the sur-
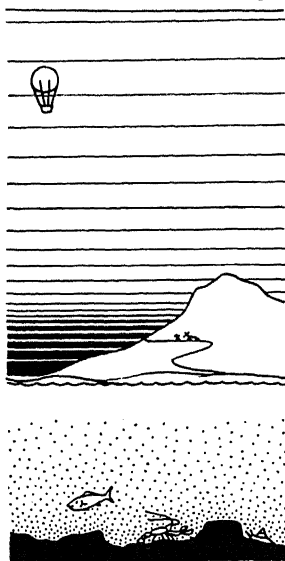
FIG. 47. Particles in liquids—and colloidal suspensions—as well as in gases are more closely packed together in the lower regions because of the "weight" of those above.

face of the earth and an increasing rarefaction as, by balloon or mountain trail, we reach the higher levels.

If the Brownian movements were greatly decreased, for example by strong cooling, the colloidal particles should settle out under the pull of gravity; and so they do in those nonaqueous colloids which can be cooled sufficiently, below the freezing point of water. If the force pulling them out of suspension were

FIG. 48. Rotor and solution chamber of ultracentrifuge. (After Svedberg. From C. L. A. Schmidt's *Chemistry of Proteins and Amino Acids,* by courtesy of the author and of Charles C. Thomas, Publisher.)

greatly increased, then likewise they should separate out. This can be done quite easily by whirling the solution, for the centrifugal force so generated, acting exactly like gravity, tends to throw out heavy particles more than it does light ones. Modern apparatus, with high-tensile steel alloys, oil or air bearings, and turbine-type drive, permits such speeds of centrifuging that the force of gravity is multiplied a million times. In fact, the only limit to the speed at which a rotor may be whirled is the strength of its materials; when it spins fast enough the steel disc itself explodes into fragments. Fortunately the steel, and even the quartz windows in it through which one may watch a solution as it rotates, are strong enough to do the trick.

Place in the transparent chamber near the edge of the disc a colloidal solution, say a solution of red hemoglobin in water. The colloidal particles dancing about are spread uniformly among the water molecules, and the red color seems homogeneous throughout. The oil pressure lines are slowly opened and the steel rotor begins to turn in its heavy steel case. As it gains speed, the friction of the air alone would soon boil the solution, were the chamber not rapidly evacuated. It attains the speed of an ordinary motor, 30 revolutions a second, 1800 per minute, while it is still getting under way. Slowly the tachometer needle creeps to 100 a second, 500, 1000, and hovers finally at 1300 a second. At this speed, an ounce of matter

Fig. 49. Progressive settling of blood pigment, photographed while being centrifuged at high speed. (After Svedberg. From C. L. A. Schmidt's *Chemistry of Proteins and Amino Acids,* by courtesy of the author and of Charles C. Thomas, Publisher.)

weighs many tons. A beam of light passes through two holes in the case, between which the quartz windows and the red solution flash by, and into a measuring telescope. The flat red surface stands perfectly still before the eye.

Then, gradually, with odd languor amid this prodigal display of motion, the solution undergoes a change, seeming to creep of its own volition. The surface of the liquid remains apparently stationary in the field of

the telescope, but the red color moves down and a thin layer of clear water appears under the surface. This layer widens until a definite zone of colorless water is sharply separated by a line of color from the red solution below. Before our eyes, the heavy colloidal hemoglobin particles are being pulled slowly from among the water molecules.

The faster the centrifuging, the more rapidly do they move and the more completely are they thrown out. If several different kinds of colloidal particles are present together, each one descends at its own rate; in fact, it has been possible by this method to measure quite accurately the size, weight, and even shape of the particles of a great variety of colloids. The scientist, as always, returns to experiment and observation to verify his theories. Theoretically derived equations which deal with falling bodies can be applied to the observed rates and distances of fall of colloidal particles; and the weights and sizes so calculated for these particles agree yet again with those obtained by quite different methods.

Now that we know with considerable certainty the dimensions of a whole colloidal particle, what of its internal structure? When, as is the case for many proteins, all the particles settle under centrifugal force at exactly the same rate, they must be identical in size and weight. When, further, the calculated weight agrees with the weight of a protein molecule as given by its atomic formula, it follows that each colloidal particle is a single protein molecule. The type of chemical analysis considered in the last chapter tells us how individual atoms or groups are attached together in the molecule, but gives only an occasional clue as to how they are sprawled out in space. The fatty acid that is composed of a continuous chain of eighteen carbon atoms, for example, might be extended as a fine thread or coiled into a tight ball, and individual atoms attached to each other might be close together or rather far apart.

Surely we should be satisfied with the information we have, glad to take the pounds and let the pennies go. No, scientific curiosity and ingenuity are not yet exhausted, and in an amazing number of cases it is possible to state within one one-

hundred-millionth of an inch exactly w̶h̶e̶r̶e̶ ̶m̶o̶l̶e̶c̶u̶l̶e̶s̶
are placed in a molecule. To be sure, this is senseless in the
case of molecules in the gas or even in most liquid forms, since
there they squirm and twist and are continuously bending their
atoms about so that no fixed position exists. But in solids,
whose molecules have been rigidly packed by the attractive
forces into row after row and tier upon tier of regularly spaced
hordes to make a crystal, these positions have meaning and
can be measured.

Ordinary light is too crude a tool for this job, for the atoms
are closer together than the crests of two succeeding waves of
light so that, even if we could magnify them enough by some
scientific hocus-pocus, they would be only blurs in such light.
X-rays, however, are extremely short light waves and differ
from visible light as do the quick short ripples on a pond from
an ocean wave. Just as the small ripples pass right through an
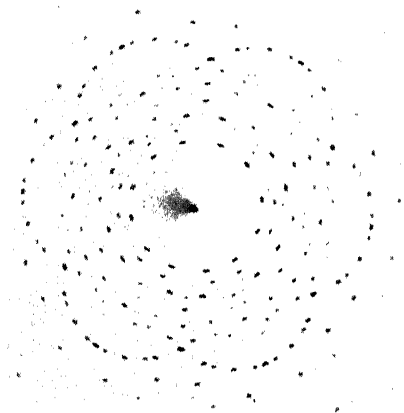
FIG. 50.   Photograph of X-rays scattered into a characteristic definite pattern of
spots by passing through the regularly arranged molecules of a crystal of
beryl. (From Bragg and Bragg, *The Crystalline State*. By permission of the
author and of the Macmillan Company, Publishers.)

open piling that breaks up the wave, so X-rays pass through masses of molecules that block or reflect ordinary light. But even the ripples are partly reflected where they hit single posts in the piling and, depending on the direction from which they come and the distance apart of the posts, the oncoming and reflected ripples make a standing pattern on the water surface. In the same way, X-rays make certain reflected patterns
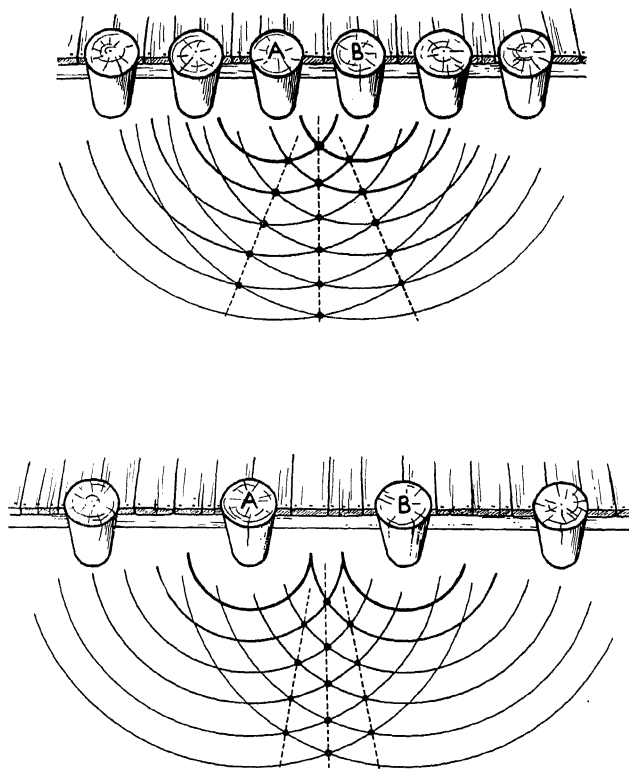
FIG. 51. Water waves reflected from the piling of a wharf may form a constant pattern on the water surface. For a given size (length) of wave, the lines of the pattern formed will vary in position with the spacing of the posts. Thus, in this diagram, the farther apart the posts, the more nearly parallel are lines connecting the intersections of the reflected waves. By measuring these lines the spacing of the piling can be calculated. In the same way, from X-ray refraction patterns, the spacing of atoms and molecules in a crystal can be calculated.

FIG. 52. Photograph of "standing" waves in a dish of water showing patterns. (From H. B. Lemon's *From Galileo to Cosmic Rays*, University of Chicago Press.)

which depend on the distance apart of atoms and on the direction from which the rays come relative to the direction in which the atoms are lined up.

Such "X-ray analysis" of crystals has, in recent years, given as accurate blueprints of their structure as any architect's plan; and now, with still improving methods, it is possible to use the same procedure to analyze smaller and less regular masses of molecules, the semi-crystalline solids and the colloidal particles present in or obtained from protoplasm. In this way, checked of course by other quite different methods, it has been possible to show that the fatty acid molecule, though bent or curled in the liquid state, is stretched its full length in the solid. And, to anticipate a bit, since molecules in a surface behave as though in the solid state, even though the surface is one between two liquids, the molecules of fatty acid on the surface of an oil drop suspended in water are also rigidly extended.

A colloidal particle of a single protein molecule is roughly spherical, with amino acids in a chain irregularly intertwined or else bound into a latticework; but when a number of thread-like protein molecules are built together, like a sheaf of straw, into a larger particle, a micelle, then they also are more or less completely stretched out into their regal length. Micelles, in
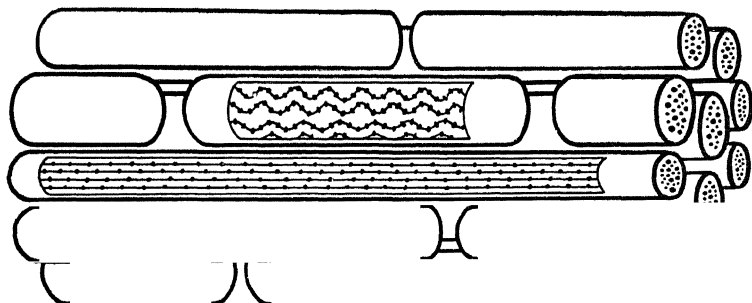
FIG. 53. Micelles built of long protein molecule threads. When the threads are normally wrinkled, as in the upper micelle, the substance can be stretched by pulling them straight, as in the lower one.

fact, are regularly rod-shaped, often quite long and slender, and the length, perfectly visible under the microscope, is that of a single protein molecule. In some cases, the separate atom groups in such a molecule form a wavy fuzzy thread, in others they are straight, as if taut. A solid built of micelles of the first type is very elastic, like hair (or rubber), since the individual protein molecules can be stretched by taking up the slack between the atoms. Solids of the other type, such as silk fibre, are quite inelastic, for there is no slack to take up in the molecules; and if pulled sufficiently hard to elongate them, they never resume their initial length, for the separate micelles or even the separate molecules within a micelle are pulled past each other and remain in equilibrium in the new position when the pull is released. The protein micelles ranged along the length of a muscle cell respond to changes in the chemical state of its protoplasm. The protein molecules stretch out or wrinkle up as the ions in the water about them are altered; and here perhaps, in the general properties of protein molecules and colloidal structures, is to be found the clue to muscle contraction and the movements of living beings.

Electrical Affinities

So much, then, for colloidal particles and micelles as individual bodies, and for their internal organization. What of their
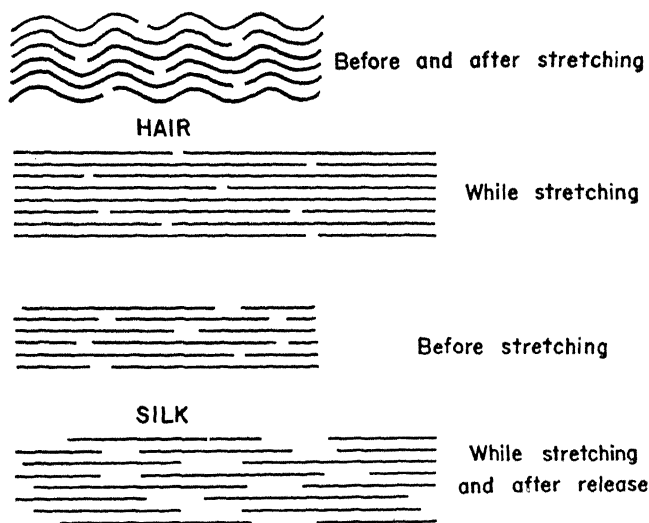
FIG. 54. The protein chains in hair are wrinkled: they straighten out when stretched, but curl again when released. Hair is therefore elastic. The chains in a silk fiber are already straight, and silk is not elastic. When it is stretched by a force strong enough to slide the protein molecules past one another, it remains elongated after the pull is released.

relation to each other and to the surrounding liquid? In human society, paradoxical though it seems, both of the adages, "Like attracts like" and "Opposites attract each other," seem to have a certain validity; and the same is true of molecular society. The situation is, perhaps, a little clearer in the latter case, so that the paradox may be resolved.

The fundamental rule is, of course, that opposite electrical charges or fields attract one another, while like ones repel. Since each atom is composed of a positively charged nucleus with negatively charged electrons swarming about it, these forces are important in maintaining the integrity of the atom. In individual isolated atoms, the number of electrons is always such as to exactly equal the positive charge on the nucleus, so that the whole is neutral. Because of certain stresses and asymmetries within the atom, however, each has certain tendencies

to alter its neutral state. Moreover, these are different for each species, that is for the atoms of any particular element. Some, like oxygen or chlorine, tend to acquire extra electrons, making the atom negative; others, like hydrogen or sodium, tend to lose them, making the atom positive; while still others, more nearly balanced at the top of the energy hill, may roll equally well down the two sides and either take on or lose electrons to become correspondingly negative or positive.

The reasons for these particular atomic prejudices are now well understood, and actually a great part of modern chemistry, including the explanation of the valence and other chemical properties of elements and compounds, derives logically and simply from these few underlying arrangements. All the elements fall into certain groups, depending on the weight and charge of the atomic nucleus, giving the extraordinarily fruitful periodic table of valence. Here we must not inquire further into the origins of this atomic etiquette, but look rather at some of its consequences.

When hydrogen and oxygen atoms, each neutral, are brought together, electrons pass from the hydrogen to the oxygen and much energy is released, if under control as heat, otherwise in violent explosion; and the positive hydrogen atom and negative oxygen atom, because of their opposite charges, are firmly held together in the new molecule of water. Chlorine and sodium in the same way pass through violent nuptials to form sodium chloride. The larger and more complex molecules are all built along the same plan, the electrical force between separate atoms holding them together. Carbon's special role in organic chemistry results from its intermediate electrical position, and the consequent ability of long chains of carbon atoms to exchange or, better, to share electrons with one another in a sort of hermaphroditic existence.

The attachment between two carbon atoms in a molecule, say of alcohol, differs from that between sodium and chlorine atoms in one of sodium chloride in that in the latter case an
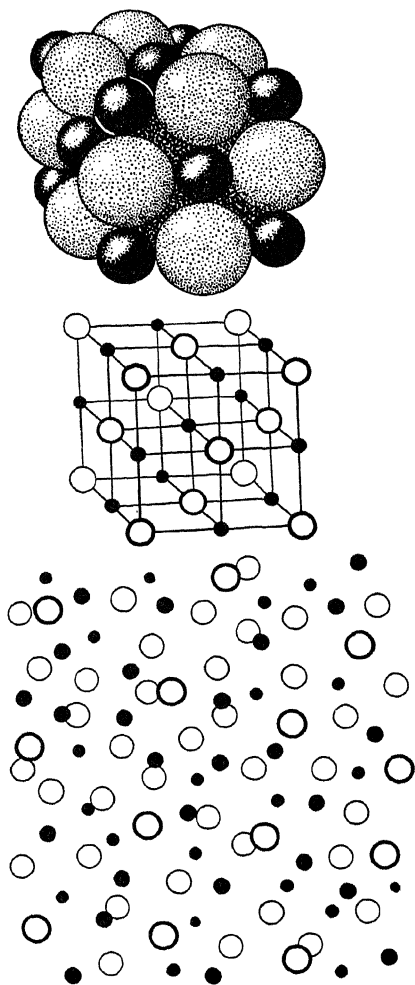
FIG. 55. In a crystal of sodium chloride the atoms of sodium and of chlorine are so alternated that each atom of one kind is surrounded by atoms of the other kind. Such "lattice" (top and middle figures) does not contain separate molecules of NaCl. When the salt is dissolved in water (below), the charged atoms, or ions, all separate from each other; but those of one charge still tend to cluster about their opposites. (Partly after Bragg and Bragg.)

electron has definitely passed from the shell of one atom into that of the other, whereas in the former the electrons are shared by both atoms and remain in their respective shells. Consequently, the sodium and chlorine atoms are held together only by the electrical attraction between them; the carbon atoms are held, electrically to be sure, but by a more subtle structural necessity as well.

If somehow the electrical pull could be lessened, the sodium and chlorine particles, one with a positive and the other with a negative charge, would fly apart and go their own ways; but the carbon atoms would still remain attached. One way to cancel the attraction of a particular sodium to a particular chlorine particle is to surround each with a whole cloud of the opposite kind. If, for example, a positively charged sodium atom is surrounded more or less continuously by negative chlorine, it

will be no more attracted to one chlorine atom than to another. And, in fact, an ordinary crystal of table salt has just such alternate layers of the two types of independent units. When dissolved in water, which we have previously noted has the power of diminishing attractions, the separate atoms dance away from their rigid positions and go their individual ways. Scattered through the solution are single positive sodiums, Na^+, and negative chlorines, Cl^-, each still more or less vaguely surrounded by a halo of the opposite type. Such charged units are called ions. A molecule of sodium chloride, then, breaks up when dissolved in water into a sodium ion, Na^+, and a chloride ion, Cl^-.

"Ion" means wanderer and such charged molecular fragments earn their name by wandering through a solution in a fixed direction under the influence of an electric current. When the negative pole of a battery is connected to a metal plate at one end of a tube containing pure water and the positive pole to a plate at the other end, very little happens. Only one water molecule of every ten million is present as the ionized particles H^+ and OH^-. The neutral molecules are not pulled either way by the charged metal plates and practically no current flows through the liquid. Adding sugar or other molecules which do not ionize does not change the situation; but if a little sodium chloride is dropped in things happen.

The Na^+ ion is repelled by the positive plate and attracted by the negative one and, though it continues to jostle about among water molecules, the steady electrical pull causes it to migrate gradually to the negative side. The Cl^- ion similarly moves toward the positive plate; and these two streams of charged units amount to a flow of electric current through the solution. As soon as the salt is dissolved a considerable current flows, while before there was essentially none. Further, sodium ion accumulates at one end of the tube and chloride at the other; as shown by direct chemical measurements. Clearly such observations give evidence as to which molecules ionize, to what extent, and into which ions—even of how many charges each possesses.

$\oplus$ = Na

$\ominus$ = Cl

$\oplus$ = H

$\ominus$ = OH

$\oplus$ = K

$\ominus$ = HCO$_3$
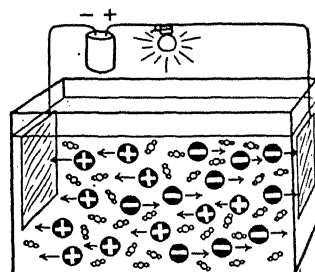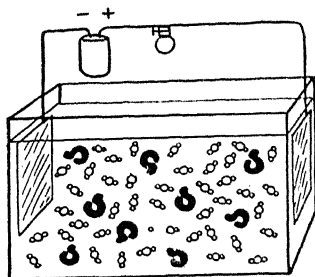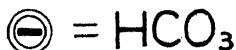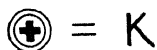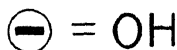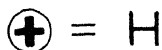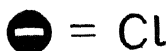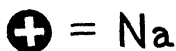
Fig. 56. When a battery and light bulb are connected to electrodes dipping into a solution of sugar in water (above), nothing happens. There are no (or a negligible number of) charged ions present to carry the electric current and complete the circuit. When table salt is dissolved in water (below), however, each salt molecule ionizes into a positive sodium and a negative chloride ion. These move through the water under the attraction and repulsion of the charged electrodes and so serve to carry the current. The circuit is complete, current flows, and the lamp glows.

At the left are shown the symbols which will be used to indicate various ions in the figures to follow.

Many molecules similarly ionize in water, though in most cases one of the ions formed when a molecule splits is a charged group of several different atoms rather than a single one. Thus, nitric acid, HNO_3, forms hydrogen ions, H^+, and nitrate ions, NO_3^-, Lye forms Na^+, and hydroxyl, OH^-, ions, calcium chloride, $CaCl_2$, gives two Cl^- ions and the doubly charged Ca^{++}. Acetic acid,

$$H-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle H}{|}}{C}}-\overset{\overset{\displaystyle O}{\diagup\!\diagup}}{C}-O-H,$$

an organic compound, also ionizes to give an H^+ ion and an acetate ion,

$C_2H_3O_2{}^-$. But note that none of the atoms directly attached to a carbon atom, whether it be another carbon, an oxygen, or a hydrogen, becomes separated from it. Carbon, by sharing electrons with other atoms, keeps them firmly bound to it to give a permanent combination. If any atoms split from the molecule, they come, so to speak, from its periphery.

An organic molecule, say a fatty acid or a protein, dissolved in water might give off one or a few hydrogen ions, leaving behind a large negatively charged ion containing the overwhelming bulk of the molecule. Proteins, because of their amino groups, may also give off hydroxyl ions under appropriate conditions, leaving behind positively charged protein ions. Protein molecules, therefore, can act like weak acids or weak bases by giving off either a few hydrogen or a few hydroxyl ions. If the hydrogen ions are removed from solution to form water, by adding a strong base (concentrated hydroxyl ions) to combine with them, the protein molecules will eventually all be brought into the form of negative ions. Similarly, if strong acid (concentrated hydrogen ions) is added to remove the hydroxyl ions, the protein will end up as large positive ions.

It all comes down to this: molecules dissolved in water may either remain intact, as in the case of many non-ionizing organic compounds, or may split to a greater or lesser extent into two or several charged ions. All salts and strong acids and bases are rather completely ionized in solution. Weak acids and bases, including most of the organic molecules which fall into one of these groups, are only slightly ionized; but if they are changed to salts, as by substituting Na^+ for H^+ or Cl^- for OH^-, they become highly ionized.

Now what has all this to do with the relations of one molecule to another and of a colloidal particle to the pygmies swarming about it? Everything; because the same electrical forces that hold atoms together to form the molecule also keep the separate molecules from flying apart. In that perpetual battle against the momentum of the dashing units, which tends to carry them in all directions away from their fellows, this is the

force drawing them back together. When the tendency to fly apart, because of increased movement, becomes greater than the attractive forces, a solid or liquid mass of molecules literally explodes into a gas, or boils. And the molecules in the gas, if not held in some container, continue to scatter widely. The greater the attraction between molecules, the higher the velocity needed to send them flying, the higher the temperature at which the substance boils.

How is it that an atom or a whole molecule which is electrically neutral, as all except ions are, may still exert an electrical pull on another neutral atom or molecule? The answer is again in terms of organization. Though the entire unit is neutral, the nucleus and electrons of which it is built are not homogeneously placed; hence one portion will be more negative and another more positive, just as a small bar magnet is magnetically neutral but still has two opposite poles. At a distance, of course, the electrical influence of such a molecule will be nil, even on an ion, since attraction and repulsion of the two charged poles of the molecule counteract each other. But at short range, where the distance from one molecule to another may be less than that between the ends of one, as in the case of long stretched-out molecules, then the asymmetry of charge can lead to very strong attractions between the two.
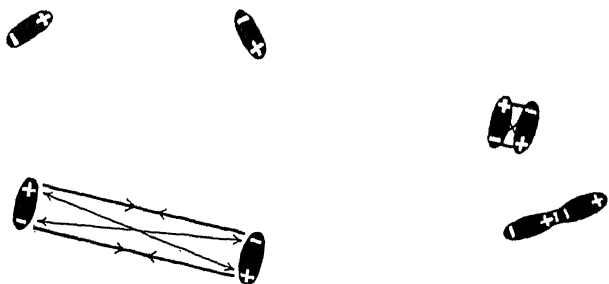
FIG. 57. Two "polar" molecules at a distance have about equal strengths of attraction and repulsion. But when they are close, the attraction between opposite poles is greater than the repulsion between like poles.

Here again we are touching a vast field of science which, though only recently plowed, is already yielding rich crops of understanding of the organization of matter. Physicists and physical chemists are now measuring the shape and size of the electrical fields of atoms with the same confident precision with which they earlier measured atomic sizes and positions.

All right, then; among atoms and molecules opposites certainly attract each other. Now what of the attraction of like for like; why do birds, or molecules, of a feather flock together? Here the explanation is quantitative rather than qualitative, though the same attractive forces are involved. Obviously, where a stronger force is drawing two molecules together, they are more likely to achieve contact than when the force is weaker. Two strong magnets will attract each other more than can two weak ones and more than will a strong and a weak one. If two species of molecules, one with a strong electrical field, the other with a weak, are originally mingled together at random, they soon become completely separated into pure clumps of one or the other kind. Those with the stronger field attract each other more than they do the other variety, and when they accidentally come together in their tortuous travels, they remain locked in an electrical embrace. The molecules with weaker fields, by a sort of exclusion, likewise form their own group. When two kinds of molecules have fields of similar strength they may remain well intermingled.

It is really not unlike the playground at school; the big boys and the little boys get separated into their own groups even though the urge for any one youngster to stay with big brother may be greater than his desire to play with his fellows. If fat and water molecules are mixed together, the water molecules, with their strong fields, aggregate and the fat molecules, with their weak fields, likewise aggregate, until a sharp layer of oil appears above the layer of water. One may shake and stir and splash the two together till fine oil drops float throughout the

water, but as soon as the agitation ceases like joins like and
the two layers promptly reappear.

Surfaces

At last we are ready to face the question we really want an-
swered. If colloidal particles are jigging about among mole-
cules and ions of water and other dissolved substances in a
solution, why do they not join together in the course of their
inevitable collisions, until gradually the whole mass of them
has clumped and jelled out? Why do not the colloidal particles,
or for that matter other dissolved molecules, join together, like
with like, and separate from the water? With the dissolved
molecules the story is simple. The water, by decreasing the at-
traction between molecules, throws the balance between the
holding forces and the scattering movements in favor of the
latter, exactly as does heat. When a lump of sugar has dis-
solved in water, the sugar molecules are in the same state of
free and independent motion as are the water molecules in the
water vapor produced by boiling a drop of the liquid.

The story for colloidal particles is more complex, more inter-
esting and, from the point of view of living processes, incom-
parably more important. The particle has a surface, its con-
stituent molecule or molecules may ionize hydrogen, hydroxyl,
or other ions, which separate from the surface and wander off
into the homogeneous water solution. Conversely, ions wan-
dering about in the solution may adhere on the surface of the
particle. In either case, the whole particle, and especially its
surface, would become electrically charged, either positively or
negatively. All the particles of any one kind of colloidal sub-
stance must act alike in yielding or attaching certain ions, and
so become similarly charged. But like charges repel, and two
negative particles will push each other away just as will two
negative pith-balls and probably never come into actual con-
tact. When, by chance, two are dashing toward a headlong
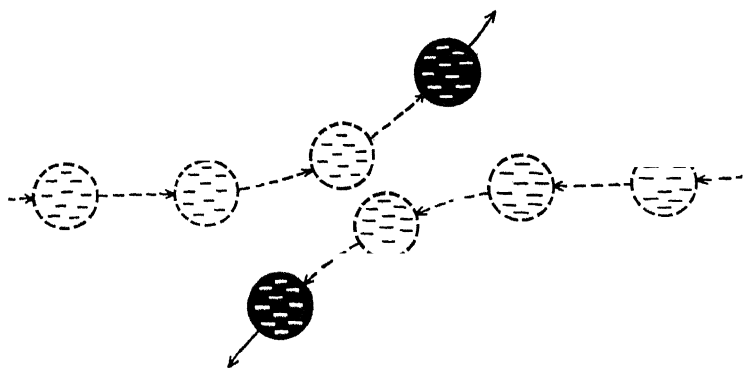
FIG. 58. Similarly charged colloid particles, though headed for a collision, deflect each other by electrical repulsion and do not collide.

collision, each will deflect the other a little from its path and the hulls slip past without touching.

Since the charge on colloidal protein particles depends on their ionization, and this in turn depends on the acidity or alkalinity of the solution in which they are suspended, one would expect to vary the charge by adding acid or alkali. Further, there should be some point at which the tendency for a protein to ionize as an acid is exactly balanced by its tendency to ionize as a base, so leaving the whole particle entirely neutral. At just this degree of acidity, the colloidal craft should be able to hit each other, cling together, form large clumps, and settle out of the solution as a solid precipitate; which is, in fact, just what happens.

A protein in alkaline or even neutral solutions acts like a weak acid, gives off hydrogen ions, and leaves the colloidal particles negatively charged. As acid is slowly added, the hydrogen ions are, in a sense, driven back onto the protein molecules, the charge becomes less, and finally, at the neutral or isoelectric point of the protein molecules, they all come tumbling down. If still more acid is added, the proteins begin to act as weak bases, separate hydroxyl ions, gradually increase their positive charge, and again become dispersed in the liquid
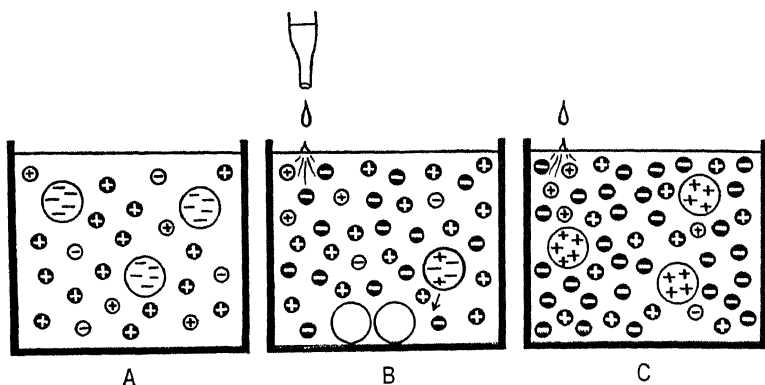
FIG. 59. At the start (A), negatively charged protein ions and a few negative hydroxyl ions are balanced by positive sodium ions and a rare hydrogen ion. As HCl is added (B), hydrogen ions combine with the protein particles to neutralize them, so that they precipitate at the "isoelectric" point, leaving in solution balanced numbers of positive sodium and hydrogen ions and negative chloride and hydroxyl ions. With still more HCl (C), the protein molecules gain more hydrogen ions (or lose hydroxyl ions), become positively charged, and are "redissolved" as colloidal particles. The electrical balance is now established by positive ions of protein, sodium and hydrogen, and negative ions of chloride and hydroxyl.

as separate colloidal particles. The exact degree of acidity at which a particular protein becomes neutral is characteristic for that individual, so that if acid is added to a mixture of proteins in the same colloidal solution, each kind tends to precipitate out at a separate point. This method has been widely and successfully used to separate and purify some of the important proteins of cells, including the all-important group of hormones and enzymes.

The charges on protein micelles can also be removed by adding other substances, which form unionized compounds with the protein ions. A great variety of such substances exist, including particularly compounds of the heavy metals, such as mercury or lead; and adding them to a protein solution promptly precipitates out a lead or mercury protein compound which is unionized, uncharged, and insoluble. The experiment

works equally well in a pure protein solution in a test tube or in the highly mixed protein solutions in cells. This is unfortunate, for these metals and similarly acting protein precipitants produce their severely poisonous effects by promptly coagulating the protein in living tissues.

When bichloride of mercury is swallowed, the cells lining the mouth and stomach are coagulated and killed; and, unless prompt and effective therapy is administered, layer after layer of these dead cells slowly disintegrates, ulcers form, blood vessels rupture, and the wall of the stomach may be completely perforated. It is not surprising that a standard treatment for mercury poisoning, other than the obvious procedure of removing the poison from the stomach as rapidly as possible, is to feed large amounts of egg white. Mercury *will* coagulate proteins, and what could be more reasonable than to offer it egg albumen on which to act and so divert it as much as possible from the living cells.

Still another important property of surfaces is their tendency to keep small molecules firmly attached to them. The electric fields around molecules in the solid state, tending to keep them together, we have seen to be greater than those between molecules in the liquid or still more in the gaseous state. As molecules form a solid, they come together vigorously, since the molecules with strong fields do exert a greater attraction on each other than do those with weaker fields. Once the molecules settle into a solid matrix, however, they are no longer migratory but remain fixed in position. Two separate particles of solid, therefore, cannot flow together to make a single whole; though if surfaces could be produced smooth to molecular dimensions, two solid masses with such surfaces would automatically become one continuous whole when placed together. Some approach to this can be made with carefully smoothed steel discs, and when two such polished surfaces are simply placed together it is beyond human strength to pull them apart. At the surface of a solid, then, there still remain these strong

attractive forces reaching out for additional molecules to hold; and since additional molecules of like kind are not available, other molecules or ions will be caught and held "adsorbed".

Every surface, except one in a strict vacuum, is covered by a film of such adsorbed molecules, sometimes no more than one layer thick. The ordinary surfaces in air—a sheet of paper, a windowpane, the top of a metal desk, our own skins—are all delicately clothed in a continuous layer of molecules of water, oxygen, and nitrogen caught from the air about. Everything is, so to speak, wrapped in molecular cellophane. Solids surrounded by liquids adsorb layers of liquid molecules in similar manner, and the colloidal particles are no exception; in addition to their other complexities, they carry with them a thin or heavy skin of water. This skin is a further aid in keeping the particles from colliding and sticking together, and if other molecules dissolved in water come to replace the water molecules adsorbed on the particle surfaces, this also changes their dispersion and other properties.

Turning from colloids of the protein type, often called water-loving or hydrophile colloids, to the water-fearing or hydrophobe colloids of the lipin type, we find the same forces effective and the same principles applying, though the details are interestingly different. Fat molecules cling together rather than mix with those of water, and a particle or droplet formed of a mass of them is practically uncharged, since these molecules do not ionize, and it does not acquire a layer of water molecules adhering to its surface. The particles can, therefore, easily collide and, having done so, stick together. If one could obtain a kind of molecule which would plaster over the surface of such a droplet and at the same time be compatible with water, then the oil molecules in their tiny skins would behave like hydrophile particles. The needed molecules were discovered, by practical experience, when wood or bone ash was mixed with fats. The lye in the ash frees the fatty acids from the glycerine of the fat and then forms salts with them. The soda or potash salts of fatty acids are our ordinary soaps.

The long tail of the soap molecule is simon-pure fat; the head, containing the acid group and sodium or potassium, almost all ionized, far more closely resembles water in its electrical condition. Soap molecules placed in water have contented heads but repudiated tails and, rather like a long row of pigs at a watering trough, they form a layer on the water surface with each individual tail pointed away from the mass of water molecules. When soap is dropped in oil, conversely, the molecular tails are quite at home but the heads are socially out of it. Now what should happen when soap is added to a vessel containing both oil and water? At last the poor half-breed molecules have found a niche, for a layer of them can lie contentedly between oil and water with their tails in the oil and their heads turned to the water. If the mixture be shaken so that new surfaces are formed between masses of oil and of water, another layer of soap molecules promptly appropriates the happy homes. In this way, droplets can be fragmented into smaller and smaller sizes, until at last true colloidal dimensions are reached. The
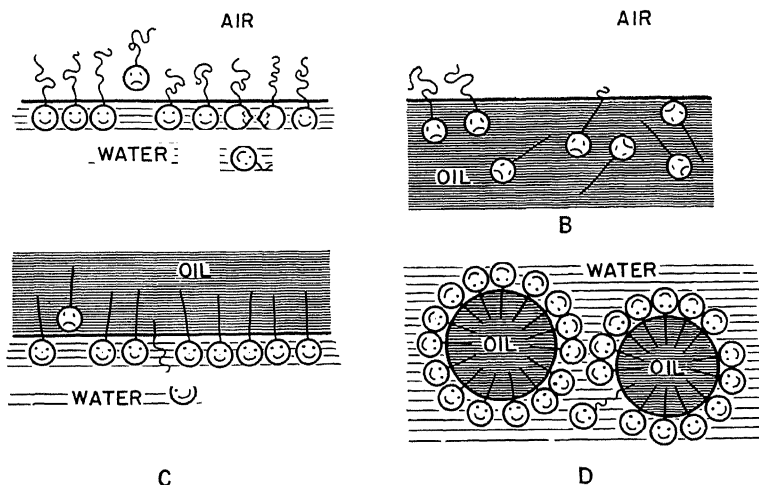
Fig. 60. The "heads" of soap molecules are "contented" in water, but their "tails" are not (A). In oil, the reverse is true (B). At an interface or boundary of oil and water, both parts of a soap molecule are at home (C); so soap films surround drops of oil in water (D).

separate particles, each surrounded by this film of soap, no longer coalesce. They are now, like the proteins, charged and covered by alien molecules which keep them from mixing.

The Greeks used olive oil to cleanse the fatty secretions from their skins. This proceeded smoothly enough, like dissolving like, but required much oil and left a good layer behind. We wash ourselves and utensils with water, and add soap to "cut" the grease. The soapy water, on rubbing, easily forms a suspension with the fat, which washes away. A colloidal suspension of fat in water, an emulsion as it is commonly called, forms easily with the aid of soap or of other molecules behaving similarly. Acetic acid molecules, the acid of vinegar, are partly like fat, partly like water, and with their aid and plenty of stirring, a French dressing results.

Let us return to the layers of oil on water with soap molecules between, and again shake. Both oil and water are liquids and can easily fragment into drops. There is no reason really for assuming, as we tacitly do, that the emulsion would end up with droplets of oil dispersed in water. Why not just as well
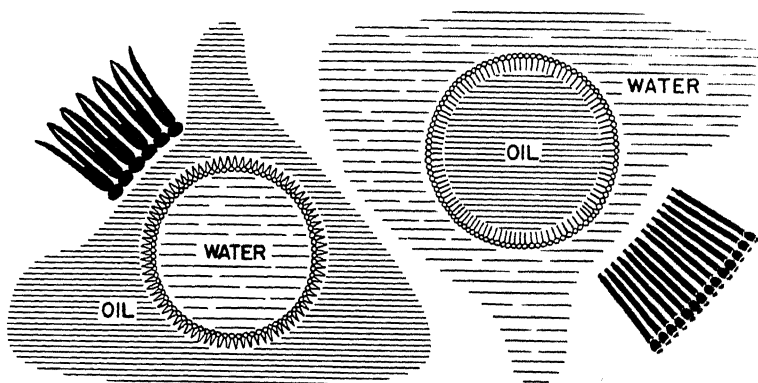
FIG. 61. A layer of calcium soap, with two fatty acids to each molecule, curves toward the "head"—like clothespins—and gives an emulsion of water droplets in oil. A sodium or potassium soap, with one fatty acid, forms a layer which curves away from the "head"—like matches—and gives an emulsion of oil droplets in water.

droplets of water dispersed in oil? In the former case, the layer of soap molecules around the oil droplet has to curve with the tails a little closer together than the heads; much as happens if a palisade of matches is laid with each match as close as possible to its neighbor. The row gradually curves away from the heads since these are larger. An ordinary sodium or potassium soap acts this way, and the emulsion resulting is always of oil droplets dispersed in water. But in a calcium soap two fatty acid molecules are attached to each calcium atom, a little like the two legs of a clothespin, and the tails tend to spraddle. Whether for this reason or some other, it is a fact that the palisade of soap molecules curves in the opposite direction with calcium soaps than with potassium ones, and leads to the formation of an emulsion of water droplets in oil.

We have already seen something of the tremendous importance for the normal performance of cells and protoplasm of a proper balance of salt ions. Here, in the shapes of molecules and their arrangement on colloidal surfaces, is at last a partial explanation of how potassium stops the heart or excites the brain. What should happen to the oil and water if, instead of a pure calcium or sodium soap, we add just such a proper mixture of the two that their effects are equally marked? Either the soap layer should not curve at all, and the oil and water drops
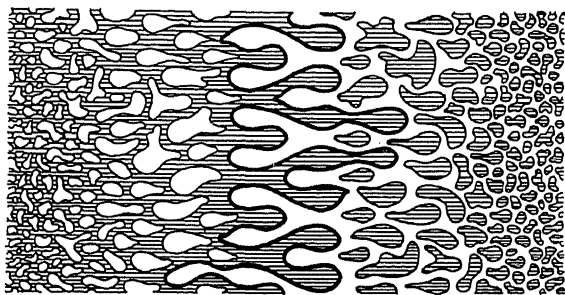
Fig. 62. Change from oil droplets in water, to "critical" state of intertwined oil and water, to water droplets in oil, as the proportion of sodium to calcium ions is altered.

be flattened out again into two complete layers, which does happen in certain conditions; or the soap surface might curve, now this way, now the other, to form a more or less continuous convoluted boundary. Then there would be neither oil in water nor water in oil, but an intricate intertwined membrane of soap separating oil from water.

Along the coast of Maine, the sinking shore line has left hill-tops as innumerable islets, each bit of land completely surrounded by continuous channels of water. If this be likened to an emulsion of oil in water, then the Minnesota lakes, each circumscribed by a continuous land barrier, must be an emulsion of water in oil. And the Florida Everglades, with long tongues of half-floating land interpenetrated with bayous of partly land-locked water, would represent the critical emulsion which is simultaneously oil in water and water in oil.

Again some surprising new properties result. Separate colloidal oil droplets floating in water can slide to and fro past one another about as easily as water molecules themselves. Even colloidal droplets of water in oil can glide by with little more difficulty than the individual fat molecules have in slipping past one another. Oil is more viscous than water to be sure, so such an emulsion flows less readily, but the difference is not great. In the critical emulsion, however, there are no continuous layers of water or oil in which the dispersed particles can slip along, only the soap surface is continuous. And molecules in a surface, as we have seen, act like molecules in the solid state. Any change in shape of such an emulsion requires the bending or tearing of these surfaces, rather than the easy slipping of like molecules upon each other. So the emulsion acts like a fragile solid, holding its shape rather than flowing freely. One need only think of a good thick mayonnaise, a slightly more complex emulsion of water, lipoids, and air, to visualize the result.

Colloidal proteins show a very similar behavior. So long as the protein molecules are dispersed in water, the colloidal solu-
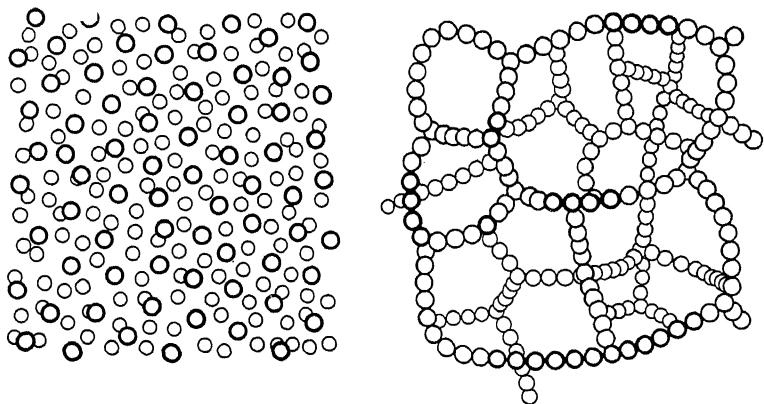
FIG. 63. Colloidal particles in the sol state (left) are separated from one another like islands in a lake. Such a sol is therefore a continuous liquid and flows easily. When the particles stick together in interlacing strands in the gel state (right), the mass is more to be compared with lakes separated by strips of land. Such a gel, then, is a spongy solid and, though soft, holds its shape.

tion is quite fluid. If they partly coagulate, however, under one or another of the conditions that enable the particles to come together, but are not allowed to bunch so much as to completely separate from the water, then a sort of network of touching protein molecules may spread through the entire bulk. Enmeshed in such a microscopic cotton batting, separate lakes and inlets of water remain trapped. The colloidal solution is no longer a liquid but a soft solid; in fact, a typical jelly. In the fluid state such a colloidal solution is known as a sol, in the more solid state as a gel; and by shifting the conditions—kinds of ions, degree of acidity, temperature, etc.—to and fro, such a mass may be brought at will into the sol or gel state.

This is nicely seen when solid gelatin, a protein, is dissolved in hot water to form a sol. The fluid gels on cooling and the jelly sols again on warming, over and over again. If too much water is present, so that the colloidal particles are relatively far apart, even when they decrease their motion in the cold they still remain well separated and the gelatin does not gel. We

are surrounded by similar phenomena in living systems, which now, perhaps, seem less mysterious. The clotting of blood is a change of one of the proteins of blood from sol to gel. The advancing pseudopod of an amoeba is just a patch of protoplasm changed to sol and more freely flowing than the rest. A slight injury to a liver cell may change it into a coagulum, a tiny shrunken hard-boiled egg.

Membranes

At long last we are ready to look again at protoplasm: A solution in water of all manner of salts and simple organic molecules, of a few hydrogen and hydroxyl and other kinds of ions, with proteins and lipins dispersed throughout, and with here and there a large continent in the form of a starch granule, fat globule, or the like. Surely this is enough chemical and structural complexity to allow protoplasm almost any sort of behavior. No, the complexity is only beginning. Mixtures and compounds of these various molecules with one another, especially the proteins and lipoids, give a still wider range and variability. Lipo-protein micelles, with some of the properties of each substance, some of neither, are extraordinarily sensitive to the conditions at their surface. In the interior of a blob of protoplasm, surrounded by just the right ions, they float languidly about in the sol state, but at its surface, with a slightly different ionic and molecular environment, they may gel together to form a continuous skin or membrane around the whole.

Every cell has, in fact, just such a jelled membrane about its more fluid interior; and its existence depends largely on the different proportions of ions inside and without the cell. Within is more potassium and less calcium, tending to disperse proteins and make emulsions of fat in water; outside, the ratio is more in favor of calcium. The surface membrane is formed by the gelation of the protein and lipoid micelles under the influ-
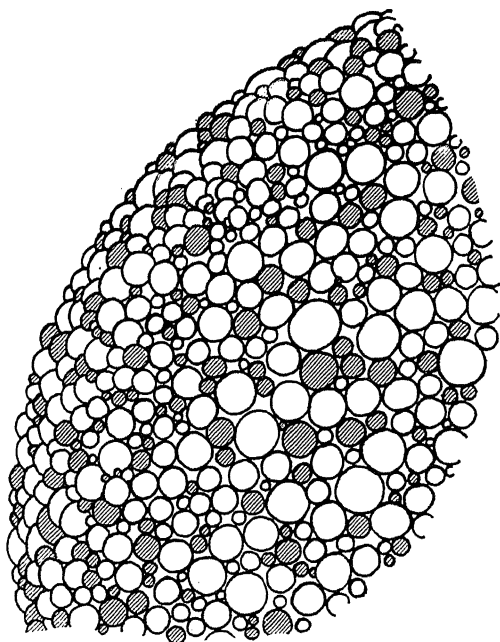
Fig. 64. Portion of the surface membrane of a cell—a mosaic of protein and lipoid colloidal particles which have gelled where the protoplasm of the interior has come in contact with other substances in the surrounding water.

ence of an excessive ratio of calcium to potassium. It is very easy to see under the microscope the changes in protoplasm induced by these salts.

Cut an amoeba, crawling about in a "balanced" salt solution, with a microscopic glass knife. The protoplasm starts to flow out through the wound. As the sol comes in contact with the new ions it gels, a fresh membrane forms, the outflow stops, and in a short time the amoeba is entirely normal again. Now add extra potassium to the outside salt solution; again make a cut. The sol starts to pour out as before but no gelation occurs. The protoplasm continues to stream out, spreads into the water and vanishes, until the amoeba is entirely dissolved. With extra calcium, on the other hand, the protoplasm hardly gets out of the cut for it does not properly gel but is actually coagu-
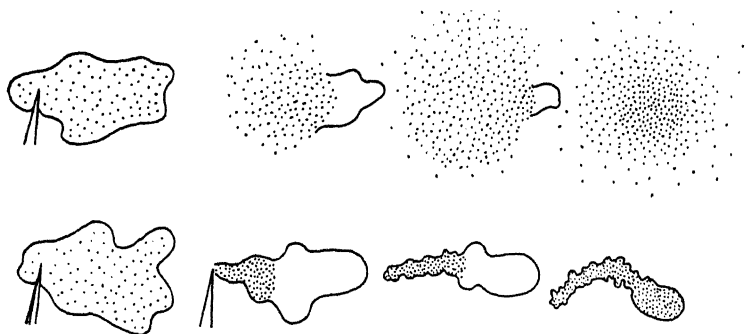
Fig. 65.   Amœba torn by a micro-needle.   Above, in a solution with extra potassium; below, in one with extra calcium. (After Chambers.)

lated. A true membrane fails to form and the calcium ions continue to diffuse in through the wound, coagulating as they go, until the tiny beast is left a shriveled, hardened mummy of itself.

The normal cell has its membrane, of gelled lipo-protein particles, which preserves the cell's integrity and keeps its protoplasm as a separate unit. Valuable molecules in this protoplasm should not escape; foreign molecules, especially injurious ones, appearing in the fluid around it, dare not be admitted. The membrane must be a barrier which is impenetrable or impermeable to such substances. At the same time, there must be an easy exchange through the membrane of all the substances concerned in the normal activity of the cell. Food and oxygen and some salts must be able to enter; carbon dioxide and water and other wastes must be able to go out again. It is extremely important to the cell that its membrane show a proper discrimination. A good watch dog must not drive away every person that comes to the door; a good membrane must be semi-permeable, offering to friendly molecules the hospitality of the cell's interior. It is truly amazing, how well this job is executed. Hundreds of substances entering our intestines daily would poison the body if they ever succeeded in passing through its lining cells. Other hundreds, often closely similar

to the others, are thriftily taken up. Some of these highly useful
and most difficult discriminations which membranes make are
not yet understood, but the great majority follow logically
enough from the character of the membrane and the sub-
stances. Indeed, some of the important exceptions, where mem-
branes permit the wrong molecules to pass, are clear conse-
quences of these colloidal properties.

The membrane is an irregular mosaic of single protein mole-
cules and clumps of lipoid molecules, with channels of water
molecules running through where the larger particles do not
fit snugly together. Through these tiny pores or holes, water
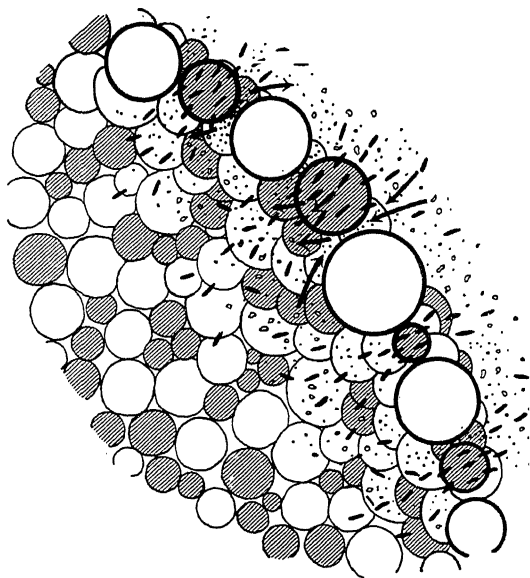
Fig. 66. Diagram of a cell membrane, cut in the plane of the paper. Lipoid
particles are shaded, protein ones not. Continuous water channels, through the
"pores" between particles, permit small molecules to diffuse between the
outside and inside of the cell. Larger molecules which dissolve only in water
are blocked by the pores; but those large ones which are soluble in fats can
dissolve into the lipoid particles of the membrane, mix with the molecules
which compose the particle, and finally emerge again on the inside as well as
on the outside of the membrane.

molecules can always pass with ease. Other small molecules dissolved in water, if not too big to shoulder through the hole, also find little difficulty; so glucose, glycerine, and amino acid molecules can enter and those of urea leave the cell. Larger molecules dissolved in water and colloidal particles, of course, are simply stopped. But molecules that dissolve in fat rather than in water, those of ether or chloroform or a whole host of other organic compounds, enter the cell easily even when they are far too large for the watery canals. These molecules can dissolve in the lipoid blocks of the membrane, can mix freely among the clumped fatty molecules, and can then wander out again, to the inside of the cell as well as to the outside.

Such molecules do, in fact, tend to accumulate especially within the lipoid part of the membrane, to dissolve in lipoid droplets throughout the protoplasm, and even to be absorbed on the surface of protein particles partly to replace their usual water film. By covering and "clogging" the surfaces of cells and of colloidal particles, such substances profoundly affect the life processes of the cell. These are the narcotics and anesthetics, whose molecules butter over the important surfaces and so retard the activities of cells. The brain cells, which are very rich in lipins, are especially easily depressed by these drugs, otherwise they would not produce anesthesia before other vital actions were dangerously slowed.

Ions, small though they often are, have a more complicated time with the membrane. Some charges are left on the micelles that compose the gelled mosaic. If the charge happens to be negative, then negative ions approaching the membrane from either side would be repelled before they got into the water channels, while positive ions could easily pass. But if positive ions passed from inside out, and no negative ones could cross, the solution left behind would soon have such an excess of negative ions that the positive ones would be attracted back again. Only when the positive ions leaving the cell are

balanced by an equal number, though often a different kind, entering can any real ion movement occur. It is for this reason, for example, that certain cells live happily in pond water but are injured when placed in salt solutions with ion proportions more closely resembling their own protoplasm.

If the membrane is positively rather than negatively charged, then, of course, negative ions can exchange but not positive ones. The charge of the membrane, like that of its protein constituents, is determined largely by the acidity or alkalinity of the surrounding solution; so acidity can control the permeability of cells for ions. Red blood cells in the fluid blood plasma normally have the colloids of their membranes charged slightly positively. Negative chloride and carbonate ions, but not the positive ions of sodium or potassium, can exchange between the
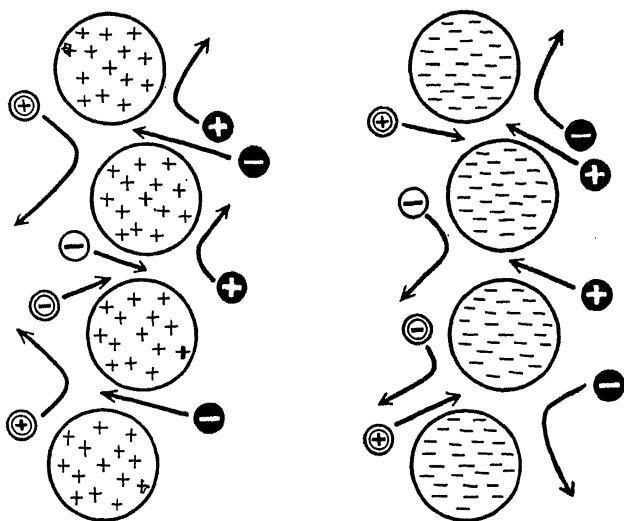
FIG. 67. At left, portion of the membrane of a normal red blood corpuscle with positively charged protein particles in the mosaic. Positive ions (potassium inside, sodium outside) cannot penetrate since they are repelled; but negative ions (chloride and hydroxyl or bicarbonate) can exchange across the membrane. At right, the red blood corpuscle with alkali added. The protein particles have a negative charge and now permit positive but not negative ions to pass through.

cell interiors and the outside plasma. If the blood is made slightly more alkaline, so that the protein charge becomes negative, then the sodium and potassium can pass but no longer the chloride and carbonate.

One other phenomenon depending on such semi-permeable membranes must be glanced at. Suppose on one side of the cell is water, on the other a solution in water of a substance, say sucrose, which the membrane does not allow to pass. The wall is bombarded on both sides by the dashing molecules, those of water or of water and sucrose respectively. A certain fraction of the water molecules that reach it go through to the other side, all of the sucrose ones are blocked. Now note that the system is not symmetrical, for a given area of the membrane will be hit by more water bullets on that side which faces the pure water. On the solution side, some of the hits are by sucrose instead of water.

The result is that more water molecules reach and penetrate the partition going from water to solution than do so in the other direction; an actual flow of water across the membrane occurs. Of course the same situation would hold, to a lesser extent, if the membrane separated a concentrated sucrose solution from a dilute one; water would still move from the weak solution, with more water, into the strong one, with more solute and less water. The same would occur if the solutes on each side of the membrane were of many and different kinds of molecules or ions so long as the barrier was impermeable to them. The
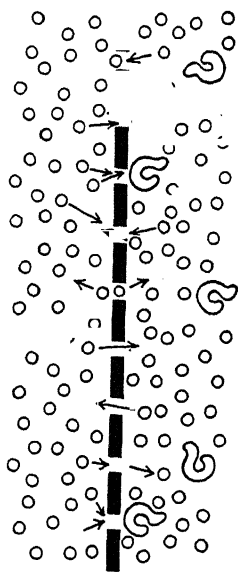
FIG. 68. More water molecules reach and pass through a semi-permeable membrane from the side in contact with pure water than from the side in contact with a solution. Water therefore moves by osmosis into the solution.
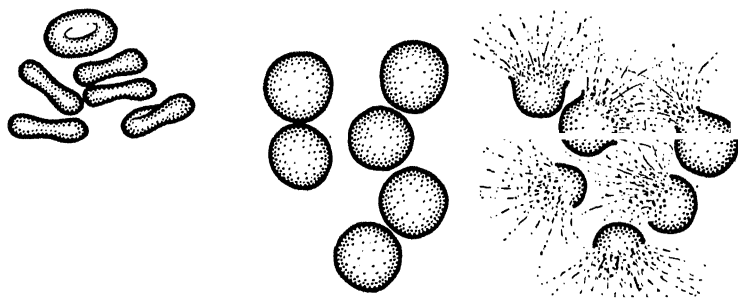
FIG. 69. Normal flattened blood corpuscles, placed in a solution of low osmotic pressure, swell up and may burst.

movement of water from a dilute to a concentrated solution (and which makes both more nearly alike) is called osmosis (a pushing through).

Since the force driving the water is really that of madly rushing molecules, it is not surprising that tremendous pressures are set up when the water movement is resisted. Water moving from the pure liquid across a membrane into a twenty per cent sugar solution can develop an osmotic pressure of twenty-five atmospheres, a pressure that can be measured only with powerful containers. No cell walls are able to stand such a strain; all rupture under far less insult.

Now we understand why the heart or any other tissue explodes when put in water instead of salt solution. Inside the cell is water with salts, sugars, colloids, and a great diversity of dissolved bits, surrounded by a continuous membrane which keeps them from passing easily or at all. Placed in pure water, the water enters the cell by osmosis, stretches the membrane as the globule bloats, and finally bursts it like an overdistended balloon. Try the experiment with two dried prunes, which still possess some of the semi-permeable cell membranes. Put one in water, the other in a strong sugar solution or syrup, and let both stand over night. That in water swells greatly as water osmoses in. The one in syrup swells little if

any, for the sugar outside of the prune skin competes with the other solutes inside and no great osmotic pressure difference develops.

This, then, is an outline sketch of the organization of protoplasm. Its extreme sensitivity, its manifold activities, and its complex properties follow continuously and often clearly from the simple properties of atoms and molecules. Its colloidal particles, its surfaces, its charges, in short its elaborate and fine organization, make it the most complex stuff we know. Its behavior is extraordinarily labile and rich, though it is not mysterious, and many of the most impressive attributes of living things turn out to be a direct expression of molecular forces.

We have not, of course, exhausted the architecture of the cell. In fact, we have hardly looked beyond that of protoplasm itself, still seen under the most powerful microscope as a homogeneous, almost structureless, fluid. But protoplasm is in turn organized into the numerous microscopic structures visible within the cell, often of bewildering and beautiful intricacy, and always bearing important functions. The nucleus and its chromatin; the centrioles and their asters; cytoplasm with its mitochondria, granules, and tortuous Golgi bodies; the special fibrils, droplets, and partitions; the membrane and, often enough, further appendages of it such as cilia; these structures or organs, unique in size and distribution for each kind of cell though common to all, are built of colloidal protoplasm and, with it, constitute the living unit of all animals and plants.
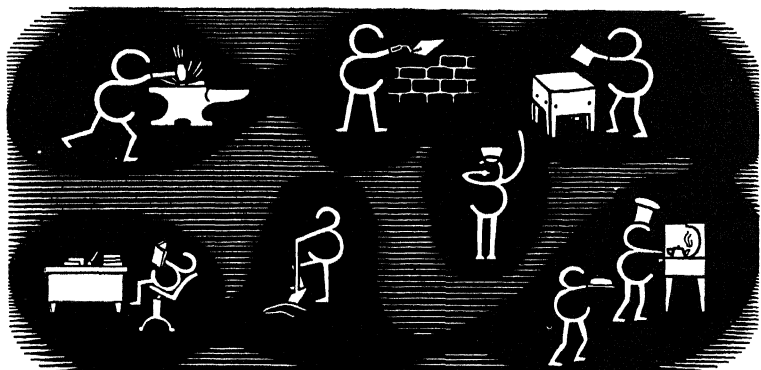
Chapter Five: Master Craftsmen—Enzymes

If some tincture of iodine is poured on the freshly cut sur-
face of a potato or if a drop or two is added to a solution of
boiled starch, a deep blue color results. This blue iodine-starch
compound is so characteristic that it can be used as a con-
venient test for the presence of starch. When this polysaccha-
ride is split into glucose, the blue color no longer forms; but
the free monosaccharide molecules form a red precipitate of
copper when boiled with a mixture containing copper sulphate.
The original starch, of course, is not a "reducing" sugar so does
not give this test. By these two simple procedures, then, it is
easy to test for the presence of starch or the glucose of which
it is built, and so to follow the changes of one into the other.

Starch boiled for many hours in water shows little or no
change. It is only when strong acids are added and the boiling
long continued that the starch slowly disappears. But if some
saliva is added to a little starch solution, in a very few minutes
the starch is gone and glucose is present. This makes a really
simple kitchen experiment. Put a lump or two of laundry starch
into an ounce of tepid water and stir until a good opalescent
solution is formed. Dip out a spoonful and drop into it a very
small drop of tincture of iodine; still better, dip a glass rod first
into the iodine and then into the sample of starch. Then collect

one or two spoonfuls of saliva, by simply spitting into the spoon, mix this with the remaining starch solution and let it stand. Every two or three minutes, dip out a spoonful of the mixture and again test it with iodine. The deep blue color formed at first is paler after a short time, then a red appears, then a pale red, and finally no color at all results. Starch has been completely "digested" by the saliva.

What is it in the saliva that is so much more powerful than strong acids and long boiling in splitting the starch molecule? The slimy mucus? The water or salts? Or some special molecule dissolved in the mixture? A simple test is to heat the saliva for a moment, even without bringing it to a boil, and then repeat the experiment. The mucus, the salts, and the water have not been affected by this treatment, but the saliva now has no more effect on the starch than a like amount of water. Something has been destroyed. By other chemical or physical maneuvers, this critical substance can be separated almost completely from the other constituents of saliva. It turns out to be a protein compound, present in the original saliva in very small amounts indeed. It has been named ptyalin (for saliva, *ptyalon*) and it is, of course, an enzyme.

How does an enzyme work? A few molecules of one mixed with millions of molecules of the substance upon which it acts, the substrate, rapidly and expertly change them, one after another, and emerge unscathed from the microcosmic holocaust; a veritable Cyrano de Bergerac, one against hundreds, felling them all. How does an enzyme work? In many ways its action is reminiscent of the camel that solved an Arabian dilemma. A sheik died, leaving his property, seventeen camels, to his three sons. The will was quite explicit: the eldest son was to receive half, the second a third, and the youngest a ninth of this valuable heritage. But fractional camels are almost worthless, and a wise elder of the tribe was called upon to help solve the young men's difficulty. He said, "Allah has blessed

me with many camels and for the love I bore your venerable departed parent I will add from them one to the herd he left you." The problem was now untangled; for the eldest son received nine camels as his half of the eighteen; the second, six; and the youngest, two. Nine, six, and two, however, added up to the original seventeen, and the wise patriarch took back his camel, along with the blessings of those who had benefited. That camel was the enzyme.

The molecules of most substances react together very slowly indeed. Were this not true, our world could scarcely continue as it is, since everything would be rapidly changing into something else. Such interactions as do occur easily have already taken place; and it is only as man has been able to take apart the stable substances that were produced and again reform the highly reactive compounds, that we have come to know them. Iron cannot exist long in nature, only iron rust; and dynamite is not a natural mineral. Yet many of these stable substances are potentially able to react quite vigorously. Some sort of chemical friction or obstruction alone prevents.

Sugar, for example, can burn, can combine with the oxygen of air, if this resistance is sufficiently lowered by heating the sugar until its molecules become partially shaken apart by their frantic movements. Even in the cold, a lump of sugar may perhaps slowly oxidize, but it would be difficult to find this out except by detecting a perceptible decrease in the amount left after a sufficiently long interval. What do we mean by "slowly" in this connection? One molecule oxidizing every hour, or every minute? A very simple calculation shows that if a million sugar molecules disappeared every second, day after day, year upon year, for the length of a century, the most sensitive chemical methods we now possess would be unable to detect the loss. No wonder that the breakfast laid out in Tutankhamen's tomb was still peacefully waiting there thirty-three centuries later. The enzyme molecules accelerate the

reaction, enormously, so that when some of them are present a few hours or seconds witness as great a change as would centuries without them.

How does an enzyme work? An enzyme molecule combines with that of a substrate, or those of several substrates, to form a new compound; a highly reactive and unstable one which almost instantly splits up again, but not into the portions from which it was formed. The enzyme emerges intact, but the remaining molecules produced are different from those of the original substrate. They are the "reaction products," the job is done. In many cases, enzymes may not even form definite compounds but merely adsorb onto their surfaces the reacting molecules. There, under the influence of powerful forces, they alter their atomic arrangements, or exchange atomic partners, and emerge in their new form. All the magic of Circe is involved; crew after crew of sailors march in and grunting herds of swine run off. But the magic here is only that of electrical attraction between atoms and molecules, in broad principle no different from, and hardly more complex than, those which build atoms into molecules and molecules into solids.

Chemical catalysts all work more or less in the same way; reactions will occur at surfaces, of large single molecules or colloidal particles or tiny granules, which do not occur in their absence. Much of modern chemical industry is built upon the use of such catalysts; from the fixation of nitrogen, through the manufacture of sulphuric acid, to the combination of lipins with hydrogen which turns ill-smelling and unstable fish or vegetable oils into white, tasteless, and enduring shortening and oleomargarine. The catalyst in a majority of these reactions is some finely divided metal, colloidal nickel being a favorite. The finest and most efficient catalysts of all, however, when the right ones can be found, are bacteria and yeasts and molds and their relatives. Alcohol, vinegar, lactic acid, and a host of

other important chemicals of industry are now made almost exclusively through the labors of these microorganisms.

Alcohol and Science

Certainly one of the oldest chemical reactions that man has domesticated is the fermentation of sugar to alcohol. How man first happened to drink a mash of wild berries or fruit which had stood too long in the warm sunshine we will never know, but the physiological effects of alcohol certainly resulted and were apparently welcomed; for fermented juices were well known at the dawn of history and a whole series of beneficent gods have been created to receive man's gratitude for them. Early domestication of plants was dedicated to thirst as well as to hunger, and the grape became the symbol of joy and plenty. For several millennia man was content to grow, ferment, and drink the juice of grapes without inquiring into the miraculous transformation of ambrosia into nectar. It had been found, to be sure, that the wines produced in certain regions were especially satisfying; and that "seeding" a fresh mash with a small amount of a well-fermented one caused the same bouquet to be produced. It was also well known a century ago that occasionally the whole mess went sour, in every sense of the word, and vinegar formed instead of wine. The going sour of wine vats spread like an epidemic through whole districts.

Such a disaster came upon the vintners and beer-makers in northern France in the middle of the past century. When the old-timers had called upon their memories and imaginations, when all their "certain" remedies had failed, and when a serious economic collapse was well on its way, an extreme measure was resorted to. A young chemist, recently come to the University of Lille, was consulted. Louis Pasteur had no ready-made solution. But, having scored one striking success in

discovering two kinds of crystals of the same substance by observing through the microscope, he again made use of this powerful tool. The reward was prompt.

When he placed a drop of the scum from a healthy vat on the microscope stage, he saw it crowded with tiny spheres, whereas a similar drop from a "diseased" vat showed a mass of even smaller rods. This was true on every test; and further, if some of the sour mash was added to the healthy one, he saw that as the wine turned to vinegar the spheres turned to rods. Yeasts and bacteria were not unknown, but it was Pasteur who clearly showed that sugar was changed to alcohol by a yeast, to acetic acid by a bacterium. This discovery, and the practical measures of control that it clearly indicated, solved the economic difficulties of the growers. It also initiated a host of scientific disputes and uncertainties.

Did the living yeast break up the sugar molecules, or did these organisms produce some special kind of molecule which had this remarkable virtue? Must the yeast be living? Was it really living? Did it form a living molecule? Was fermentation, in short, a question of biology or chemistry? Pasteur and his followers championed the importance of living processes and showed, indeed, that the yeast was alive and grew and could be seen to bud and reproduce. But Liebig threw the weight of his chemical eminence on the other side and, with his cohorts, produced evidence that killed yeast could also ferment sugar, so that living cells were not necessary. A scientific Franco-Prussian war raged for a quarter of a century with major victories on both sides and, for such is the nature of scientific wars if of no others, an enormous increase of knowledge and benefit resulted.

Fermentation did not depend on living yeast and yet it did; for if a mass of killed yeast was added to a test tube of sugar this was changed to alcohol, but a vat of grape juice had to be seeded with live yeast to form wine. Another young man finally solved the difficulty. Buchner, only at the turn of the

century (he was killed in the World War), proved everybody right. By powerfully squeezing the bodies of huge numbers of yeast cells, he obtained part of their protoplasm in the form of a juice, which had no cells and was clearly just a complex chemical mixture, but which powerfully fermented sugar. This active juice or the substance in it, however, was formed only by living yeast cells. The life process was necessary to form the catalytic molecules, but once formed their activity was a matter of straight, though complex, chemistry. The active substance was very noncommittally called something "in yeast (leaven)" or, translated into Greek, enzyme. The word rapidly came to be used for the whole class of catalytic substances found in or produced by living organisms, and the more specific term for the fermenting enzyme of yeast has survived as zymase.

Zymase, however, was far from the first enzyme discovered, for the digestive enzymes of gastric juice were known a century earlier. There was, of course, no question that food was "digested" somewhere between mouth and anus, for even when large chunks of partially chewed meat or vegetables were wolfed down, they did not reappear as such in the feces. They were hardly recognizable even in the stomach contents when regurgitation accidentally occurred some hours after a meal. The burning question of the day was, how is digestion brought about? Did it depend on the mechanical grinding and squeezing action of the muscular stomach and intestines, carrying on over a longer time and more thoroughly the work so well initiated by the teeth; or was there in addition some chemical action by the materials accumulating in the stomach, which leave a disagreeable sour or bitter taste when they accidentally come up through the mouth?

Spallanzani made a frontal attack on this problem with the greatest directness. If the gastric juice is important in digestion, he reasoned, then lumps of solid food exposed to its action, yet protected from the stomach movements, should still

digest. He put such bits of food into tiny rigid cages, attached a thread, and swallowed the capsule. After varying lengths of time, and with considerable personal discomfort, he retrieved the cage via the thread and noted its contents. They were progressively softened and diminished and, when enough time was allowed, finally vanished entirely. The stomach juices, then, circulating about the food, could slowly digest it.

Well then, once the stomach had secreted this juice, was the stomach itself at all necessary, or would the same digestion take place in a test tube? Again Spallanzani proved himself an intrepid explorer. He swallowed sponges attached to a thread, heroically hauled them up again, and squeezed the gastric juice they contained into a test tube. A piece of meat or egg dropped into it was digested as before. Digestive enzymes acting especially on proteins, for example the well-known pepsin, were thus demonstrated; but it was not until the later work that the tremendous implications of this discovery began to unfold. These enzymes, though formed in cells, were then discharged by them and worked as simple chemical agents. A great widening of the scientific horizon occurred with the realization that, even within cells during their normal life activities, comparable substances acted in similar ways.

Enzyme Properties and Poisons

Any particular enzyme is composed, like every other pure substance, of its unique molecules. In perhaps a dozen cases now, these have been obtained so nearly pure that they have formed crystals; an important test for homogeneity, since only identical molecules will settle down in the proper patterns. Pepsin from gastric juice; trypsin, another protein-digesting enzyme thrown into the small intestine from the pancreas; urease, which rapidly breaks urea into carbon dioxide and ammonia; and insulin, the antidiabetic hormone of the pancreas; are among those successfully crystallized. In each case
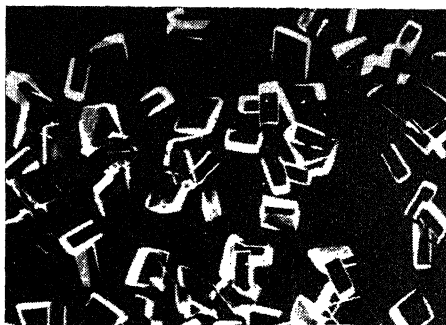
FIG. 70. Microphotograph of crystals of a form of trypsin, a digestive enzyme and a protein molecule. (After Northrup. From C. L. A. Schmidt's *Chemistry of the Proteins and Amino Acids,* by courtesy of the author and of Charles C. Thomas, Publisher.)

the substance has turned out to be a protein, with or without some particular additional atomic groups built into the molecule. In many other cases, though the final purification is lacking, it is possible to be quite certain about much of the composition of the enzyme molecule, and surely in most cases— some scientists believe in all—it is protein in part or whole.

Those enzymes which are composed solely of protein offer, in a sense, the greatest problem for, with our incomplete knowledge of these complex substances, it is impossible to identify
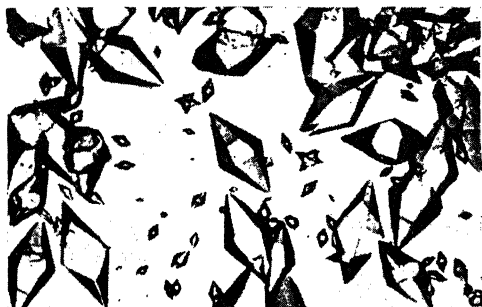
FIG. 71. Microphotograph of crystals of pepsin, a protein-digesting protein molecule. (After Northrup. From C. L. A. Schmidt's *Chemistry of the Proteins and Amino Acids,* by courtesy of the author and of Charles C. Thomas, Publisher.)

that particular arrangement of atoms which confers upon each its catalytic activity. The special chemical groups combined with, or even loosely attached to, the protein in the other class of enzymes are far simpler and many have been completely identified. Indeed, in several cases, the exact atoms responsible for the enzyme action have been singled out and the chemical basis for this behavior established.

One important group of enzymes in cells, that having to do with the fermentation of sugar and probably including zymase itself, contains in the protein part of the molecule sulphur atoms, each one linked to one carbon atom. When the other valence of the sulphur is attached to hydrogen, giving the sulphydryl group (—SH), the enzyme is active. When, instead, two of the sulphur atoms are linked together to give the —S—S— combination, a change which is easily brought about by oxygen, the enzyme is quite inert. We are not surprised to find, then, that certain poisons such as arsenic, which combine with sulphur, destroy these enzymes and stop fermentation. The less dramatic but far more important fact, that fermentation occurs best when oxygen is absent and may stop entirely when plenty of oxygen is at hand, finds a similar easy explanation. In the presence of oxygen, the inactive —S—S— form of the molecule is produced and the enzyme for fermentation is not available. When oxygen is excluded, the enzyme molecule is reduced to the active —SH state by other substances in the cell, which would ordinarily have combined with and reduced oxygen, so fermentation proceeds. The enzyme is in no way injured by its change in either direction, so that as often as the cell is deprived of oxygen it enters into active fermentation, which stops promptly when oxygen is again supplied.

Another substance, almost universally present in cells, is a small edition of these sulphur enzymes. Glutathione, a tripeptide, contains the amino acid, glutamic acid (hence the prefix, gluta), a still simpler one, glycine, and a sulphur-containing one, cysteine (thione, for sulphur). It is, therefore, a small piece of

a protein and it falls far short of the fermenting enzymes in its catalytic properties. Nevertheless, it does have the sulphydryl group which can also be oxidized to —S—S— and again reduced to the original form, and it does enhance certain important chemical reactions in cells.

The substance vitamin C, an essential constituent of normal diets, is taken up also by practically all cells. It is closely related to glucose but more acidic and, since its absence from the body leads to the severe symptoms of scurvy or scorbutus, it is known as ascorbic acid. Ascorbic acid, like glutathione, can be oxidized and reduced again with relative ease and has a definite significance in each state. In fact, ascorbic acid is one of the cell substances that can react with glutathione to oxidize or reduce it, so that an important connection exists within the cell between these two very necessary substances, and together they help to control other chemical changes.

By far the most important enzymes in all cells that use oxygen are those which make this use possible. Almost none of the substances in cells are directly attacked by this gas; they can be oxidized, burned, only with the aid of one or, more commonly, several special enzymes. Few branches of knowledge have seen such rapid and intellectually dramatic progress as that dealing with the oxidizing enzymes of cells. A quarter of a century ago, they were suspected to exist; today many, possibly all, of them have been isolated, identified, their chemical nature unraveled, and their mode of action explained. One of the fundamental properties of living things, respiration, is well on its way to becoming a problem of pure chemistry.

The oxidizing enzymes contain two types of special groups attached to protein. One of these has an atomic structure not too unlike that present in aniline dyes and, like them, gives rise to color. Since in these particular cell pigments the color is yellow, they are called flavins and the compound with protein a flavo-protein or the yellow enzyme. This oxidizing enzyme, though almost universally present in cells, is much less im-

portant than the other type. In the second group, the atom complex attached to the protein is rather more elaborate. It is called a hemin since it is closely related to hemoglobin and, like the blood pigment, is colored, though not always red. Of special importance, it contains one or several atoms of elements not generally present in organic molecules. These are all metallic elements, the most important being iron, the next, copper.

Several such hemin compounds are present in each cell, and they juggle oxygen molecules (or electrons) from one to the other until finally food molecules are burned. The iron atoms do the heavy work in these, much as the sulphur does in gluta-thione, for the first iron atom is oxidized by oxygen, it oxidizes the next iron, and so in turn through at least four steps. It is because cyanide forms a stable compound with the oxidized iron, so preventing its reduction, and because carbon monoxide forms a stable compound with reduced iron, preventing its oxidation, that these two gases are such deadly poisons. Even small amounts of them completely inactivate the hemin enzymes and so stop the absolutely essential respiration of cells.

Many other particular enzyme groups have been unraveled, many containing phosphate, or magnesium, or iodine, to list only some special elements; but, after all, these are carried in molecules whose overwhelming bulk is protein, and which contributes some general properties to the whole enzyme tribe.

They are, of course, colloidal and present the relatively enormous surface which is the essence of that state. It is only at this surface that the catalytic action takes place, whether it be a true temporary combination with substrate molecules or only an adsorption of them. Any agent that tends to gel, or still more to coagulate, proteins, decreases the exposed surface of enzyme molecules and renders them less effective. It is hardly surprising, then, that enzymes are almost universally

inactivated by heat, which has such a coagulating action—witness a boiled egg; and that they are not revived by cooling—again note the egg. On an entirely different basis, but also by actually clumping the enzyme molecules, ions with many charges destroy activity. Positive ions like mercury or lead are very effective in neutralizing and precipitating negatively charged colloids; and such negative ions as arsenate are similarly powerful coagulants of the positive colloids. All such substances seriously damage cell enzymes and are universal poisons for practically all living things.

Brewing is not done in metal vats, for enough zinc or tin or lead would dissolve to poison the yeast enzymes. Occasionally unbelievably small amounts of these heavy metals suffice to poison cells. At all marine laboratories, where sea plants and animals are kept in tanks for exhibition or experimentation, a continuous supply of fresh sea water is circulated by pumps. Special pipes and parts of hard rubber or synthetic plastic must be used, for if the water flows through the usual metal conduits the animals promptly die. Once a new copper gasket inadvertently placed in a pump killed off practically the entire marine population of a laboratory.

To a lesser extent than the heavy metals or their ions, but still acting along the same lines, are the common salt ions present universally in protoplasm. Calcium favors aggregation of colloids; potassium favors dispersion. Potassium might, therefore, be expected generally to improve enzyme activity and hasten cell reactions; calcium, the reverse. Over a wide range of performance this is indeed true; the respiration of many cells, for example, is augmented by small increases in potassium.

The enzyme surfaces have another, more subtle, property, with interesting consequences. Substrate molecules whose reaction is hastened by the enzyme must become attached to its surface. The total speed of the chemical change depends on the area of surface available and on the time during which each reacting molecule must remain there before its metamorphosis.

If enzyme surfaces are packed together by gelling, the reactions are slowed up. In exactly the same way, if these surfaces are occupied by molecules of a kind which does not react, and the important ones are kept away, change will be retarded.

What determines which molecules will attach to an enzyme surface? Certainly not all, else its catalytic action should be most indiscriminate, whereas actually each enzyme manifests the most finicky tastes in its chemical fare. Years ago, an analogy of the lock and key was suggested; and it is true that the spatial arrangement of atoms in one molecule may be such as to snuggle comfortably against the atomic topography of an enzyme surface while that of some other molecule may be a complete misfit. The situation would be somewhat like the
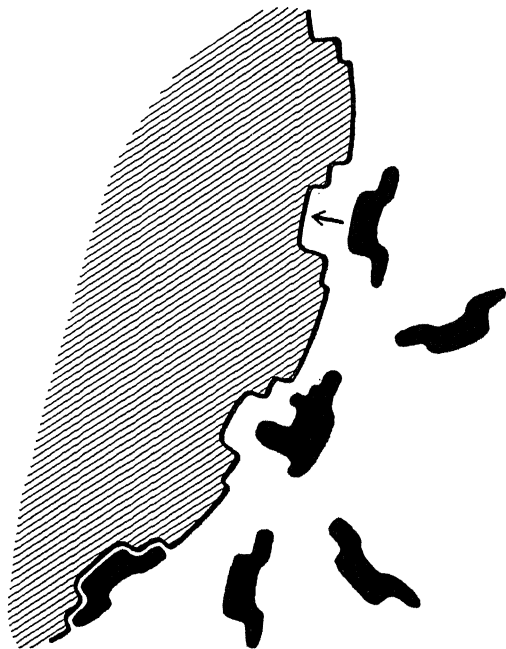
FIG. 72.   Schematic surface of an enzyme particle and two kinds of molecules in solution near it. One kind "fits" into the surface and can be acted upon. The other, a misfit, is not changed.

neat packing together of identical molecules to form a crystal, as contrasted with the unwilling and slipshod piling up of unlike ones in amorphous solids. Certainly it is common for enzymes which easily act upon, say, a sugar molecule to leave its spatially opposite twin, the stereoisomeric molecule, strictly alone. Even more important than the actual atomic positions are the electrical forces around every molecule and the exactness with which they match those at the surface. In general, the more alike two substances are chemically, the more likely are they both to attach or fail to attach to any particular enzyme molecule.

Consider one enzyme widely present in cells, which enables succinic acid to lose two hydrogen atoms and form fumaric acid. As can be seen from the arrangement of the atoms in the molecule, one hydrogen comes off each of two adjacent carbons in this reaction.
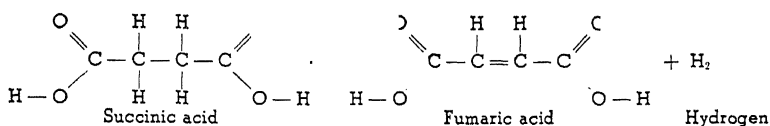
The enzyme that catalyzes this reaction, called the succinic dehydrogenase, might be expected to attach to its surface not only molecules of succinic acid but also those of the very closely related malonic acid.
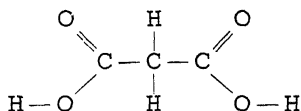
Note, however, that in this smaller molecule there are not two adjacent carbon atoms which could each lose hydrogen, so that this particular type of oxidation cannot occur. If succinic acid is added to the enzyme, it is rapidly oxidized to fumaric acid. (Of course, some other substance, an oxidizer, must be present to take up the hydrogen atoms released.) If malonic acid is added instead of succinic, no oxidation results. The malonic molecules are none the less attached to the enzyme;

for, if malonic and succinic molecules are added together, the oxidation of succinic acid is decreased. The more malonic molecules there are in proportion to succinic, and whatever the absolute concentration of the succinic, the more is the oxidation depressed. The two kinds of molecules compete for the same enzyme surface; and whatever fraction of it is captured by malonic is not available for succinic and its oxidation.

Some substances of relatively simple structure and feeble charge can attach to a great variety of enzyme molecules and are very general inhibitors of enzyme activity. In this group are ether, the alcohols, chloroform, ethylene, and, in general, the so-called narcotic drugs which, we have already learned, are readily adsorbed on surfaces. They prevent access of the appropriate substrate molecules to the enzymes and are able, therefore, to depress the respiration of almost every living cell and so to depress cell function and temporarily suspend animation. Though the nerve cells of the brain become "anesthetized" at lower concentrations than do other cells of the body, a sufficient amount of these narcotics will stop the contraction of muscle, secretion by the kidney, the beat of the heart, or the crawling of an amoeba. Just as one waxes a surface to protect it, so "waxing over" an enzyme surface with narcotic molecules keeps it also from change.

Scientists are always more certain of their ground when they can attain quantitative agreement between their predictions and results than when the agreement is only qualitative. It is more satisfying to be able to say to what extent a given narcotic will depress the respiration of a cell than to say merely that it will depress it. Let us see what can be done. A given enzyme system contains some definite, though numerically unknown, surface area. The rate at which it oxidizes, say, an amino acid, depends, among other things, on this area. If the surface were halved, so also should be the rate of oxidation. We add various kinds of narcotics to the enzyme-substrate mixture, in each case

just enough to cut the oxidation in two and so, presumably, to cover just half of the enzyme surface.

But how many molecules of a given narcotic are required to cover this particular area? That depends, of course, on the surface occupied by each molecule. The number of mosaic bits needed to cover a wall becomes larger as each piece becomes smaller. Now, the size of the molecules of many narcotics is known, and therefore the relative number of each kind which should cover a definite area. If our theory is correct, the relative amounts of various narcotics which must be added to halve the oxidation should vary inversely as their molecular sizes. Such experiments have been made, and the results are amazing-
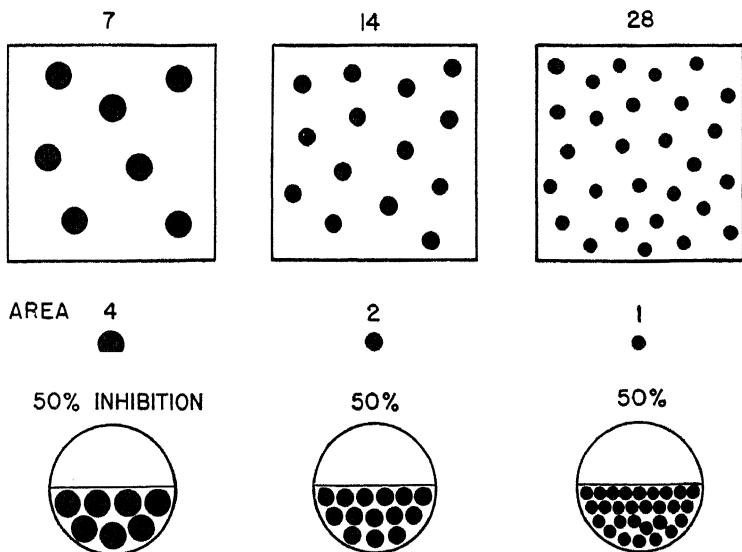
Fig. 73. Three different narcotics are used in such concentration of each as will just cut cell respiration in half. The area of each kind of molecule is known; the areas are in the ratio of 4:2:1. The concentrations required are found to be in the ratio of 1:2:4, so that each narcotic has covered the same area of cell surface, presumably one-half, when it has abolished one-half of the enzyme activity.

ly accurate. For each narcotic, the molecular size (cross-section area) multiplied by the number of molecules required comes out the same, though either figure alone shows great variation.

One other important factor requires mention in this matter of allowing substrate and enzyme molecules to get together or of keeping them apart. Many enzyme molecules are more or less firmly bound with other protein and lipoid particles as integral parts of surfaces and other structural elements of cells. Others, not actually anchored, are still penned into certain cell regions by membranes and jelled networks which interlace about them. The smaller substrate molecules are likely to be less hampered by such barriers, but still some inner cell structures might prevent their passage or they might be firmly adsorbed on non-catalytic surfaces. A single cell might, then, have plenty of enzyme and large stores of substrate within it and yet, by keeping them separated, prevent any reaction. Then chemical changes could occur almost explosively when the barriers were opened or surfaces altered, which is just what happens when a cell at rest is suddenly stimulated to activity. This story is for later telling, but we shall not be surprised to learn that when a stimulus makes the membranes of a muscle or an egg cell more permeable, there follows a burst of extra oxidations, fermentations, and other chemical events.

With the road open, the decks clear, and a strong affinity drawing them together, the substrate molecule cleaves to the enzyme surface. Here it must be so pulled out of electrical equilibrium that, on meeting its proper partner, the two react; sometimes after leaving the enzyme, sometimes while both are simultaneously attached and can effect a swift exchange of atoms before the new-born products go their way. How long does this molecular matrimony take? Longer, of course, with sluggish molecules or a feeble catalyst. But at worst the times are measured in thousandths of a second. And they are actually measured, particularly when fairly pure enzyme is available. The amount of reactant changed after a given time can be deter-

mined chemically, when the quantities are large enough, and from this is calculated the number of molecules. If, then, the number of enzyme molecules present is known, it is simple to determine how many molecules of substrate were changed by each molecule of enzyme. For one of the hemins, it has been shown that every molecule of enzyme catalyzes the oxidation of 100,000 molecules of substrate per second.

There is a record to shame any marrying parson! But, to carry on the simile, divorce is practically as easy as marriage in the molecular domain, and the enzyme parson who busily turns molecules A and B into C and D is just as skillful and rapid in changing C and D back to A and B. The enzyme is quite amoral about it. The direction in which the reaction proceeds depends on the amounts of the substances present. If there is relatively more of the first pair than of the second, then more of the first will change into the second than vice versa. But with each new C and D formed, the chance of the enzyme
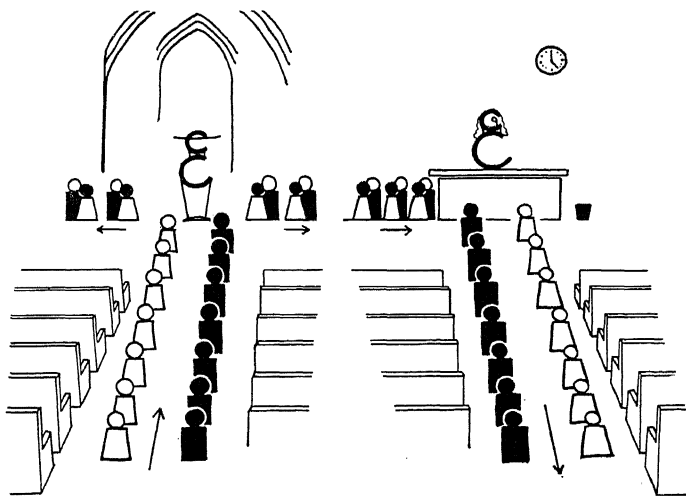
Fig. 74. The single enzyme molecule, like a preacher, can cause an unlimited number of molecules of the proper kind to unite (or react, or exchange atoms). It can also, like a judge, separate the unions and restore the original status of the molecules.

surface being occupied by these molecules increases; by A and B, decreases. There must come a time when the change in both directions is equally rapid. The mixture then comes to equilibrium and, although the enzyme continues obstinately to ply its trade and huge numbers of molecular changes occur, the total balance remains the same.

Of course, if C and D, with the aid of a new enzyme or another substance, can change in turn into other new combinations and these in turn into still others, then no equilibrium will ever result. There will be, rather, a steady change of the initial molecules through stage after stage down the catalytic reception line. Both situations occur in cells. Some reactions go to and fro, for example, the oxidation and reduction of the sulphur enzymes as oxygen is added or removed. Other reactions go inexorably forward, step by step, as when the sugar molecule is attacked by one enzyme after another until all the worth has been squeezed from it by the cell and its useless carcass of carbon dioxide is discarded.

What of the enzyme itself, through all these adventures? Like Mother Earth, upon whose surface perpetual change and interchange drive on, it goes serenely on its way. The catalyst molecule, having consummated the reaction of eight billion substrate molecules in a day, is still prepared to face a like number at the end of it. But, of course, the enzyme, itself a molecule, may relinquish the role of a player moving about the counters in his game and become a pawn itself. The succinic dehydrogenase, for example, composed of protein, is digested by pepsin as is any other protein, and so destroyed. The enzymes in a cell, potentially immortal like bacteria, are like them subject to accidental destruction: another enzyme breaks one down; an unusual molecule combines irreversibly with a second to give a new inactive compound; the wear and tear in the cell surface permits even the large molecule of another to find its way out through a temporary crevice and be lost. In such accidental ways, but never as a consequence of its prime

catalytic activity, are enzymes destroyed. Such accidents do happen, of course, yet the cell enzymes are maintained despite this constant attrition. They must, then, continually be rebuilt. Before facing the problem of their production, let us meet somewhat more intimately the various members of this family.

The Enzyme Tribe

All enzymes are formed in cells. Some, the intracellular ones, remain and carry on their proper work in the protoplasm where they originated; whereas others, the extracellular enzymes, are promptly poured out from their cellular containers to do their jobs in free solution. The entire group of digestive enzymes, from ptyalin of saliva to the juice of the small intestine, is composed of such extracellular members poured into the cavity of the alimentary canal; and the hormones, to the extent that they are enzymes, fall into this class for they are secreted into the circulating blood. Still other enzymes find their way into the blood and even into the urine, perhaps only as dying cells accidentally release their intracellular wares. Certain special enzymes, the immune bodies, to be discussed later, are rather on the boundary between the intra- and extracellular groups.

The intracellular cohorts are more numerous and more varied; and since new ones are being discovered and isolated almost monthly, it is too early to guess at the final count. Practically every one of the innumerable chemical reactions occurring within cells proceeds under the ministration of an enzyme and, even though some of these mentors preside over several reactions, the number of them required is surprisingly large.

To further subdivide the group, enzymes are characterized by the substances on which they act. Lipases attack fats; proteases, proteins; glycogenases, glycogen; phosphatases, phosphate compounds; deaminases split ammonia from amino acids or other amino compounds. Indeed, the name may be as specific as the action; maltase splits maltose to two molecules of glu-

cose, beautifully, and does nothing else at all. Enzymes are named also for the changes they produce. Hydrolases split apart large molecules into their smaller building stones with the addition of water; pepsin is a protein hydrolase which separates protein into smaller polypeptides; ptyalin is a starch hydrolase, and hydrolyzes this large molecule to maltose. The fermenting enzymes, or glycolases, of which zymase is the classic example, break monosaccharides into smaller molecules. Some of these are oxidized at the expense of others (dismutation), which results in the formation of alcohol, vinegar, lactic acid, and the like. And most important of all are the oxidases, which lead to oxidation, or their chemical equivalents, the dehydrogenases, which, by removing hydrogen atoms, produce the same result.

The suffix "ase" denotes an enzyme. The word to which it is attached describes the substance on which it acts, the chemical change induced, the source from which the enzyme was first obtained, as zymase, or a combination of these. The succinic dehydrogenase is, then, the enzyme which dehydrogenates or oxidizes succinic acid. The cytochrome oxidase is one which oxidizes this particular substance, one of the hemin catalysts, widely present in cells. Glucose dehydrogenase oxidizes this molecule; glucose "fermentase" or "dismutase," which implies the interaction of molecular fragments, or more curtly glucolase, splits glucose mainly into lactic acid. Glycerophosphate hydrolase, more briefly glycerophosphatase, splits this molecule into glycerine and phosphate.

So is described the enzyme population, within and outside of cells. Even today it is all too often true that an enzyme is known only by its performance; and when some substance added to cells is found to be chemically changed, an enzyme specifically catalyzing this particular reaction is promptly assumed and dubbed with its hereditary title. Sometimes this turns out to be an error and purification shows that no enzyme is required or that a well-known one or combination is able to catalyze this

additional change. The old enzyme may then come to bear proudly the additional emblem on its coat-of-arms.

Occasionally one particular need of a cell is satisfied by a single reaction under the control of an individual enzyme. More often, a chain of reactions, with their separate enzymes, intervenes between start and finish. "The king tells the queen and the queen tells the dairymaid," and only some time later he gets butter for his bread. These chains branch and intersect so that, starting with a given substance, the cell may end now with one, now with another product, as the activity of the various enzymes involved determines which route is traveled. The sugar molecules may reappear as lactic acid when the glycolase is performing, as carbon dioxide and water when it is not. Because of this intricate warp and woof in the chemical pattern of the cell, any chemical line traced through it is partly artificial. And also because of it the enzymes present not only modulate the speed at which changes occur but also, by accelerating one or another of the alternative reactions, they really control as well the direction of chemical flow, and so the entire metabolic past and future of the cell.

Whether the egg of a frog continues slowly to burn up its stored fats until it stales and dies, or whether, on being pricked by a sperm, it embarks upon the fascinating adventure of growth and division, leading through a labyrinthine chemical trail to another frog, its enzymes must decide. When a muscle cell, hitherto resting quietly, suddenly enters into chemical gymnastics and performs its contortion act, its enzymes are responsible. A cell of the liver unexpectedly bursting forth into rapid, chaotic growth and division to form a cancer has developed the wrong complexes in its enzyme system. Every cell is and does as its enzymes dictate. To be sure, the enzymes themselves are affected by accelerators, adjuvants, and inhibitors; they may be changed from an inactive to an active condition by ions or oxygen or other chemical means; they may be allowed access to or held restrained from their substrates by

physical barriers; all of which, in turn, are controlled by other physical and chemical happenings in the cell. Yet, in the last analysis, these preceding changes may themselves depend upon other enzymes, leaving these aristocratic molecules secure in their royal prerogatives.

How, then, are enzymes formed? For formed they must be, within each cell, during its growth and reproduction and in sufficient amounts to make good the gradual loss by wear and tear. The proteins built into them, like all the others of the cell, are fabricated within the protoplasm by a progressive linking together of amino acids, which have entered through the cell surface. An exacting crocheting, this, to add in proper sequence and position the particular amino acids called for; to form independently of one another within the tiny confines of a single cell the many different, and often enough absolutely unique, proteins that constitute its birthright.

The splitting of proteins is performed by enzymes, and since these always catalyze reactions in both directions, they should likewise build them up. Pepsin acting upon protein digests it to amino acids. Pepsin acting upon amino acids should combine them into protein, and it does—into a rather sloppy vague protein, to be sure, when man makes it perform in the test tube, but one produced by synthesis none the less. Related enzymes in the cells act more efficiently to build their proteins. The same enzymes should, of course, be able to digest them, and this is also true. If, when cells die—because of absence of oxygen or what not—they are kept warm and free from bacteria, they slowly dissolve, or autolyze, their synthetic enzymes now exhibiting a proteolytic action.

Still other enzymes must form any special attached groups, except when these come ready made in the food, and again the manner of action is sometimes fairly clear. To form the heme in hemin enzymes, one of the amino acids, proline, is chemically sculptured by splitting off atoms here and attaching them there until the proper molecule results. Four of these are bound

together, an iron atom set like a jewel in the center, and the job is done. The combination of heme and protein is a simple chemical reaction requiring no further catalyst, and even in the test tube the two can be combined or separated at will. The family of hemes, all close kin, differ only in minor chemical details, an extra hydrogen or oxygen atom usually modulating the atomic profile of one or the other.

And what a talented family it is! Probably no living cell lacks it entirely. Four heme enzymes, at least, collaborate to form the oxidases essential to most cell respiration. Another heme combined with the protein, globin, forms the hemoglobin that brings oxygen to the cells of all vertebrates and even some lesser animals. Still another heme-protein, with magnesium replacing the iron atom, is the most valuable stuff in the world, the chlorophyll of plants, and almost single-handed captures the energy of sunlight and builds it into chemical compounds. Chlorophyll reduces carbon dioxide and water to sugar, giving off free oxygen. The sugar is not only the major fuel for life processes, but is a necessary step in the manufacture of the other carbohydrates, fats, and proteins which the plant makes for its own protoplasm and for ours. The chemical complex of four proline molecules, or more correctly of four pyrrol nuclei, known as porphyrin, is almost certainly one of the very earliest chemical successes of living things. The particular porphyrin in the oxidase of cells, when reduced with two atoms of hydrogen, becomes the porphyrin of hemoglobin; when oxidized with one atom of oxygen, it becomes the porphyrin of chlorophyll. Truly this is a regal dynasty of molecules.

But let us return to the formation of enzymes. The protein and other groups of an enzyme are built by other enzymes all right, but how did these others arise; how was their protein formed? Some enzymes must, obviously, be present at the birth of any cell. It inherits them from its parent; and how a cell develops must depend in part on just which enzyme mole-

cules have been included in its endowment. This, however, is
the story of heredity and chromosomes and genes, and forms
another chapter of the story. Those enzymes originally present
in the cell must form such new ones as are needed to complete
its armamentarium. They must, moreover, increase themselves;
else with the repeated division of one cell into two they would
be diluted to vanishing. This is the now familiar story of auto-
catalysis and need not be repeated until we are ready to exam-
ine it in more detail.

Respiration Enzymes

Now for a quick glimpse at one complete chemical sequence
in a cell, that most important one of respiration. The sum-
marized story is simplicity itself—into the cell comes a con-
tinuous supply of oxygen and food substance, let us say glu-
cose. Out of the cell diffuses in turn the final products, carbon
dioxide and water. The balanced reaction is merely:
$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$
All the atoms are accounted for; the change is made with a few
strokes of pencil on paper. But in the cell, what an amazing and
intricate mechanism unfolds! A veritable old-time bucket
brigade is in action with a line of individual enzymes each
passing the precious stuff from hand to hand.
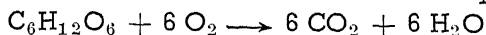
Glucose itself is not burned by most cells; it or glycogen
must first combine with phosphoric acid under the influence of
a phosphatase to form a glucose-phosphate. This may then
take on a second phosphate group, or itself be put through the
gauntlet. Let us take the simpler case. Under the influence of
its specific oxidizing enzyme, glucose-phosphate-dehydrogen-
ase, the glucose-phosphate can lose two hydrogen atoms,
giving the first oxidation product. The dehydrogenase is, how-
ever, unable to act by itself; it must be associated with a special
colloidal protein, an intermediate or carrier enzyme. Further,
the hydrogen atoms taken from the substrate must find a new
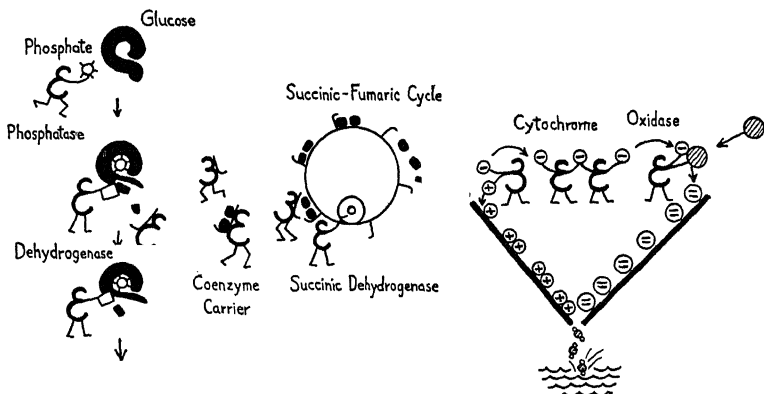
FIG. 75. The oxidizing enzymes act something like a bucket brigade or an assembly chain. Compare details with the text.

haven by combining with and therefore reducing some other molecule.

Simplest, of course, would be a direct combination with oxygen to give water or hydrogen peroxide. But even the cell has not learned this trick. Another accessory substance or coenzyme, also necessary to the reaction, combines with the hydrogen and so completes this first reaction. The glucose is now out of the picture. It is well to remember, though, that even when the adventures of these hydrogens are completed, only one step in the oxidation of the glucose has been accounted for. A dozen further steps, each different because it starts with the new oxidized product yielded by the preceding one, must likewise be completed before the glucose carcass is picked clean and is of no further chemical value. The coenzyme is now in the reduced condition and, if nothing further happened, the entire very limited supply of it would be used up in a fraction of a second and remain unable to take up hydrogen from additional glucose molecules. It, then, must pass on still further the hydrogen which it temporarily accepted.

Now succinic acid, with its enzyme, succinic dehydrogenase, and its oxidation product, fumaric acid, comes into the picture.

(It is probable that several other steps intervene here—hydrogen going to oxalacetic acid, again to a coenzyme and then to the yellow enzyme before reaching fumaric acid—but they are only more of the same thing.) The enzyme which enables succinic acid to give off hydrogen and so become fumaric acid is, of course, equally able to catalyze the combination of fumaric acid with hydrogen to form succinic. The reduced coenzyme transfers its hydrogen to the fumaric acid present, with the aid of the dehydrogenase, coenzyme being reformed and the fumaric changed to succinic acid. The same problem again remains, for soon the fumaric acid would be exhausted and cell oxidations again be stopped. Hydrogen must continue on its way. Succinic acid, its enzyme still present, will, of course, oxidize again to fumaric if some other substance will accept the hydrogen. Several candidates for this role are regularly available, but the most important is one of the colored hemin-protein enzymes, present in practically all cells, and known as cytochrome, or cell color.

Cytochrome is really not one but three distinct hemin proteins, closely related, and acting in tandem, but it will do to consider a single step. The iron atoms in this molecule, if oxidized to begin with, can be reduced and so, albeit somewhat indirectly, can take up the waiting hydrogen from succinic acid. Reduced cytochrome results and must again be oxidized if the machinery is not to be clogged. Now the cytochrome oxidase, often called *the* respiratory enzyme, since it was the first of the group clearly worked out, takes a hand. Here at last is a molecule which, when reduced, can oxidize directly with oxygen. It becomes oxidized, reacts in turn with reduced cytochrome to reoxidize that, and is itself left free to react again with more oxygen. By this devious route, the hydrogen atoms have finally reached those of oxygen and the sequence is complete.

Or is it? If two hydrogen atoms combine with an oxygen molecule, the product is not water but hydrogen peroxide, H_2O_2. The hydrogen peroxide is a strong oxidizing agent—

hence its ability to bleach hair—and in any quantity is very poisonous to cells. No, this substance must not accumulate, and two other types of enzymes now take a hand. A group of peroxidases, rather like the oxidases, carry additional hydrogen atoms to the peroxide and complete the reduction of oxygen to two molecules of water. Of course, the hydrogen comes from other molecules in the cell, substrate or one of the intermediate substances. This is the most efficient process, since the oxygen is fully utilized. But if, for any reason, peroxide is accumulating through some oxidations more rapidly than it can be utilized in these others, there is still a safety valve. The enzyme catalase can directly split hydrogen peroxide into water and gaseous oxygen. It is the escape of the oxygen produced by this re-action which causes hydrogen peroxide placed on a cut to effervesce so strongly; for enough catalase is freed by the injured cells, indeed is present in the blood itself, vigorously to break down the added peroxide.

A long story, this simple oxidation—far longer than indicated, for over twenty steps are now recognized in the breakdown of a sugar molecule and some involve the citrous acids (which make a grapefruit sour) which have not even been mentioned. Yet the process is marvelously coordinated and carried on with great dispatch. A yeast cell can break down its weight of glucose in a minute, though the actual amount of enzymes in a cell is vanishingly small, certainly less than one part of oxidase to ten million. How is it possible to describe in such detail, really in the much greater detail that is known to biochemists, processes occurring at lightning speed and catalyzed by infinitesimal amounts of enzymes, some of which have never been seen or handled? Yet these are not guesses but, in most cases at least, thoroughly established facts.

The reaction of glucose with phosphate can be carried out in the test tube in the presence of enzyme, and the glucose-phosphate isolated. Its oxidation can also be shown by strict chemical tests when the enzyme, coenzyme, and carrier are

added. The oxidation and reduction of cytochrome, even within
the cell, can be followed visually, for its reduced form absorbs
certain wave lengths of light, and possesses a corresponding
color, different from that of the oxidized molecule. If white light
is passed through a suspension of yeast cells and then through
a spectroscope, certain of the colored rays, absorbed by the
cytochrome, do not appear in the resulting spectrum but are
replaced by dark bands. It is an elementary experiment to
observe this chemical machinery in action.

Hold a test tube of yeast shaken in water in front of the
spectroscope; the dark bands of reduced cytochrome are seen.
Now bubble oxygen through the suspension for a moment.
It enters the cells, acts through the oxidase, and oxidizes the
cytochrome; the bands promptly disappear. Continue to watch
for a few seconds, as the oxygen dissolved in the water diffuses
into the cell and is gradually used up; the bands of reduced
cytochrome reappear and grow to their maximum intensity.
Hydrogen continues to be carried on by the dehydrogenases
from glucose or other food substances, until it reaches and
reduces the cytochrome; and with no oxygen to complete the
chain, there it stops. This can be repeated over and over again
with the same cells, and by so spying out their interiors, the
state of cytochrome at any moment is established.
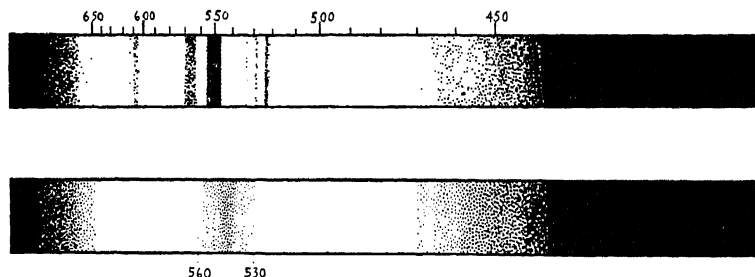
FIG. 76. Absorption spectrum of cytochrome in cells. Above, when it is fully
reduced (no oxygen present, or when the cells are poisoned by cyanide);
below, when it is fully oxidized (oxygen present, or when cells are narcotized.)
(After Keilin.)

Cell oxidations can be stopped, moreover, not only by excluding oxygen but by adding one of the various poisons that prevent its use. Cyanide and carbon monoxide block the iron-containing oxidase which mediates between oxygen and cytochrome. So far as cytochrome is concerned, the result is the same whether the oxidase is blocked or oxygen is completely absent; it can still be reduced but no longer oxidized. If, then, we bubble oxygen through our yeast, but at the same time add some carbon monoxide or cyanide, the bands of reduced cytochrome promptly appear.

The other end of the chain can likewise be halted by stopping the action of dehydrogenases; for example, by adding a narcotic to keep the substrate molecules from the enzyme surface. Chloroform added to the yeast suspension prevents hydrogen from being passed along to cytochrome which, once oxidized, remains so. The spectroscope shows no bands of the reduced form. While chloroform remains present, the cytochrome stays oxidized even if the oxygen is driven out by nitrogen or another gas, or if carbon monoxide or cyanide is added.

So, step by step, has the story been pieced together. It is far from complete. New enzymes are still being discovered in and separated from cells. Other substances, such as glutathione and ascorbic acid, are being shown to play an intermediate role, changing from one state to another and back again. Each biochemical journal contains new experiments showing how these various substances react together and with substrates, how the enzymes affect them, and what the products of the reactions are. Each kind of cell is being explored; for its enzymes are, at least in part, unique to it, and so also is the chemical road traveled through it. The jigsaw puzzle is far from complete, many pieces remain to be found, many others are not yet fitted into place; but the pattern is already clear, the picture of cell metabolism.
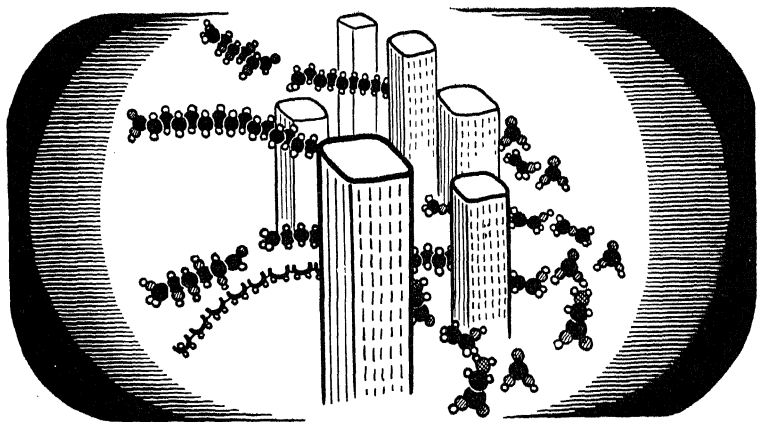
Chapter Six: Molecular Traffic—Metabolism

For two thousand years the study of life was the study of structure. Doctors were anatomists, and if they examined the body at all, it was to dissect and lay bare its parts. The structure of living things—the gross arrangements of limbs, organs, and tissues, and the far more elaborate and delicate patterns of cells and protoplasm and their constituent molecules—is, indeed, a cardinal aspect of life. Yet structure alone is dead, not living, and the constant interplay of units and behavior of the whole alone bring animation. The separation of form and function is partly artificial, partly real. A river cannot exist without its bed, nor the bed without the river. The channel is formed by the flowing water but, as formed, in turn controls the flow. The river, as an enduring dynamic entity, is not the water or the sand, but an organization, really an organism, depending both on form and flow. The dead form may persist, when the flow has stopped, as a tangible record of a past activity; but the river is no more. Considered chemically, the structural aspects of a stream depend primarily on silicates, the dynamic ones on water. A similar separation is discernible in the living cell.

The chemical motif of life as an enduring material structure is carbon, but the leitmotif of its action is oxygen. Oxygen turns the chemical wheels, drives the life machinery, supplies the energy, and animates the whole. Without carbon, obviously growth and reproduction are impossible. Without oxygen, mere existence cannot be sustained. To be sure, protoplasm has elaborated various dodges to carry on for longer or shorter periods without a direct supply of this precious element, and in a very few cases it has completely substituted some other substance, for example the closely related sulphur, in its metabolic scheme. But life in the grand panorama that is presented to view can no more be thought of in the absence of free oxygen than can a Mozart quartet without a first violin.

Living things change yet stay the same. They are dynamic but in equilibrium. Over a long enough time-scale this is not true. The cell or body grows, ages, and reproduces, with or without death as its terminus, so the equilibrium is not complete. A single life span is run through in years by man, in days by most insects, and in minutes by many protozoa and their kin. But over shorter periods, especially during the relatively long and stable period of adulthood, the organism is the same a year hence, or a day, or a minute, as the case may be.

Like Alice on the other side of the looking-glass, living things have to run rapidly all the time to stay where they are. This is part of the dynamic equilibrium which is life. A spinning top loses its upright position when the movement stops; a whirlpool can exist only while the water whirls; and one cannot conceive of a non-burning flame or a non-metabolizing protoplasm. Cells' protoplasm is continuously used up, mainly burned; their membranes are forever springing leaks; their enzymes are being lost; and the process of catabolism or chemical tearing down continues inexorably. This degradation is as continually opposed by a building up or synthesis, the process of anabolism, in order to keep the equilibrium. If catabolic processes domi-

nate, as in disease or starvation, the body wastes away and ultimately dies. With anabolism holding the whip hand, growth and development follow.

Gross Balance

It is not difficult bookkeeping to strike the balance and actually list the metabolic assets and liabilities, or the chemical turnover of a working day. Catabolism is burning and, from the amount of oxygen taken in and carbon dioxide given out by an organism in a fixed time, its rate is readily determined. For cells or organisms at rest, to exclude from the balance the extra requirements of special activities, this gaseous exchange or basal metabolism measures the speed of life. The fuel for this burning is predominantly the food taken in, and it is the energy resulting, rather than the substances, which is important to the cells. In the chemical sense, this is catabolism of the foods, not of the body, and no equivalent chemical building up is required to offset it. Yet some of the body is always participating.

Particularly is this true for proteins and related nitrogen-containing molecules. However much energy be supplied by food in the form of carbohydrate and fat, the end products of protein breakdown continue to leave the body; and the best measure of the balance between building-up and tearing-down reactions in the whole organism is the one obtained by comparing the nitrogen entering and leaving it. In the excretions of cells, or of an organism containing many cells, there are regularly present such nitrogen wastes as urea, the rather more complex creatine, or the quite elaborate uric acid. When the food contains no nitrogen at all, these substances are still excreted, a sure sign of the burning of protoplasm itself. The uric acid, especially, is a product of decomposition of nucleic acid, an essential structural component of cell nuclei. The organism is thus in negative nitrogen balance; catabolism is in the ascendant; and after a longer or shorter time death closes the chapter.

Well then, if an organism is losing say one gram of nitrogen a day, this should be balanced by adding to the food an amount of protein or nuclein or both which contains a gram of nitrogen. But it is not so simple, because now the nitrogen excreted is greater than before; not two grams to be sure, but perhaps one and a half. Some of the added protein is directly burned or catabolized, just like fat or sugar; and its amino nitrogen is wastefully split off to form ammonia and urea, which are promptly excreted without having ever entered into the structure of the cell. As more and more nitrogen is added to the diet, the gap between that taken in and that put out is finally closed and nitrogen balance is attained. With still larger intakes, at least for young cells, some nitrogen is retained, a positive balance is set up, and growth results. Even in the balanced state, of course, some of the protein is directly burned, like any other foodstuff, and it is difficult to say what fraction of this traffic eddies through the cell structure itself.

Aside from the particular nitrogen requirements and balance, the whole metabolic transfer is pretty largely a question of energy. All foods yield energy on burning, or on other kinds of chemical breakdown, and the food needs of cells are measured in terms of the necessary energy supply. The basal metabolism is a simple and direct measure of this energy turnover. Life runs at a faster tempo in the young; for young protoplasm, per unit weight, uses oxygen more rapidly than does older stuff; and a deep-seated relation exists between this chemical velocity and the speed of the whole process of living. Those cells and organisms which travel their allotted life span rapidly metabolize at a higher rate than those which trudge more slowly their appointed course. Weight for weight, the basal metabolism of a bacterium, completing its individual existence in twenty minutes, is roughly a hundred times that of a horse, which may live twenty years.

The prime requirement of food, then, is to supply enough energy to satisfy the various needs of the cell. To this end it makes little difference what chemical molecules are used or

how they are changed; so long as the total energy released is sufficient, it does not matter greatly whether one molecule yields much or each of many molecules yields little. The purchasing power of a hundred pennies is the same as that of a dollar bill, and sometimes the small change is more convenient. The second, though no less vital, requirement of food is that it contain enough of certain essential substances. Nitrogen in useful form is indispensable, and the intriguing group of vitamins belongs to this rubric also. These specific compounds are necessary to the organism not in terms of the energy they yield on burning but because they themselves or other substances produced from or by them are anabolized into the structure of protoplasm.

Reactions with Oxygen

So much for the long-range equilibrium, the low-magnification view of the chemical business of protoplasm. A closer examination throws us at once into the swirling maelstrom of catalysts, colloids, membranes, actions and reactions, the blooming, buzzing, worrying molecular confusion which constitutes the living cell. Into the turmoil come certain molecules, from it emerge others—that is clear. But by what steps and sequences and devious routes are the atoms guided into their final combinations? As in a large department store, the milling crowd pushes about, individuals enter, individuals leave, materials change hands, and to an outside observer all is the utmost in confusion—so the metabolic exchange in the cell presents a picture that seems inchoate and unanalyzable. Yet order does underlie the behavior of the shoppers and of the molecules, and the controlling conditions are simpler in the latter case. The order is apparent only when we have before us the chemists' models of molecules, the structural formulae; so these are given for orientation throughout the following sections. They are not so difficult as a quick glance might indicate.

The traffic machinery in the cell depends partly on its structure, and partly on its enzymes. By breaking the whole into its components and studying each alone, we can find the through

chemical boulevards, the blind alleys, the one-way streets, and the traffic circles; and later, by recombining the details, we can plot the molecular routes through the cell. The implied analogy fits the problem of cell metabolism in another important aspect. There may or may not be one main traffic route and easy path to travel; but there is always more than one path possible. Commercial and passenger traffic may move together or separately; single or alternate through routes may be available; temporary obstructions may require detours. The pulse of traffic rises and falls, but in the living city the automobiles do get through somehow or other; in the living cell, no less, the individual molecules do complete their reactions, by chemical highway or detour, quickly or slowly.

One route for the oxidation of glucose has already been charted. For most cells probably ninety-nine per cent of the oxidation traffic passes through much of this road. Oxygen, cytochrome oxidase, and cytochrome form a powerful system for oxidizing a variety of properly activated substrates. Other oxidizing substances, however, can substitute even for these. The yellow enzyme can accept hydrogen from certain active substrates, and so become reduced; and the reduced form can be oxidized directly by oxygen, even in the test tube, without the aid of an enzyme. Related substances are present in certain particular cells and play similar roles. They are all dyes, which can be reduced to a colorless form and then be again reoxidized. The green of a live lobster is due to such a dye combined with protein (boiling the animal frees it in a red form); and the laboratory methylene blue can act in a very similar fashion.

Methylene blue as well as cytochrome can accept hydrogen from activated succinic acid, to be reduced to methylene white. The white substance, like the reduced yellow enzyme, is directly reoxidized with oxygen. Note, however, that when methylene blue is present it offers a route for oxidations alternate to that of cytochrome and the oxidase, which cyanide renders inactive. The respiration of cells blocked by cyanide should, then, be restored by addition of methylene blue; and so it is.

Even in the whole vertebrate body, in man himself, methylene blue, though somewhat less directly, antidotes the poisonous effect of cyanide.

The oxidation of lactic acid follows a course similar to but not identical with that of glucose. Lactic acid is, in fact, one of the way stations, or more properly a very slight spur from one of the way stations, in glucose oxidation. This acid, in the presence of the lactic dehydrogenase and the lactic acid coenzyme, gives off two hydrogen atoms to become pyruvic acid,

Lactic acid   Pyruvic acid
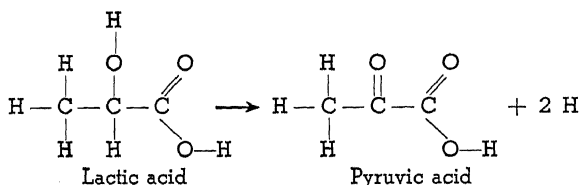
and the subsequent fate of the hydrogen atoms is again to form water with oxygen. As they jostle from combination to combination toward this goal, they probably follow the same course as those from glucose. The pyruvic acid in turn may, with the aid of an enzyme, carboxylase, and its coenzyme, lose carbon dioxide to give acetaldehyde. (Perhaps most of the carbon dioxide produced in metabolism is actually formed by this reaction.)

Pyruvic acid   Acetaldehyde
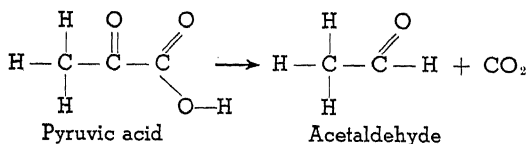
The aldehyde may then oxidize further to acetic acid or, under other conditions, be reduced to alcohol.

Acetaldehyde
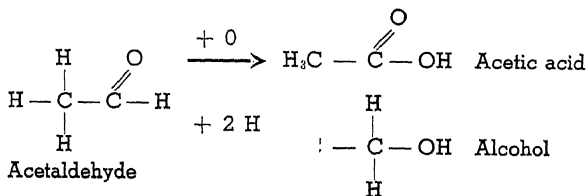
Either of these molecules may accumulate or, with the proper enzymes and other conditions, be oxidized in several stages to carbon dioxide and water.

But another, more surprising, path is open to pyruvic acid. Two molecules may join together, with the loss of two hydrogen atoms, tail to tail so to speak, to give a larger molecule containing a chain of six carbon atoms. The two ends of this lose carbon dioxide under the action of a carboxylase and, after some further oxidation, the cell is left with a molecule of succinic acid.
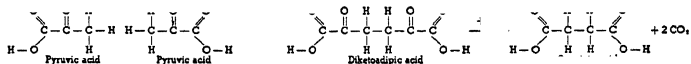
Or a molecule of the pyruvic acid may combine with a four carbon acid, and the seven carbon compound be whirled through a metabolic cycle, giving off hydrogen and carbon dioxide as a pinwheel does sparks, until the pyruvic molecule is fully oxidized and the other re-formed and ready for another spin.

Here, then, are possible connections between glucose and succinic acid, via lactic and pyruvic acids; although the extent to which such changes actually occur in particular cells is still uncertain. Succinic acid itself is, of course, easily oxidized in the presence of the succinic dehydrogenase to fumaric acid; this, with fumarase, takes on water to give malic acid; this, with its dehydrogenase, oxidizes to oxalacetic acid; and this, under the action of carboxylase, can split to carbon dioxide and pyruvic acid.
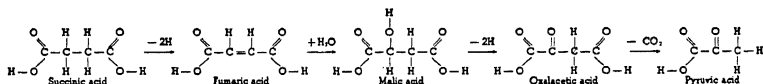
It is possible, therefore, by progressively oxidizing several molecules of succinic acid, to pass through pyruvic acid and back again to a smaller number of succinic molecules, the remainder having been completely broken down to carbon dioxide and water.

These, then, are some of the detailed changes that carbo-
hydrates and their related compounds undergo during oxida-
tion in the cell. What of the fats? The glycerine from these,
after combining with phosphate to form glycerophosphoric
acid, under the action of a phosphatase, is oxidized by losing
hydrogens from the end carbon atom to give glyceraldehyde
phosphate or from the middle one to form dihydroxyacetone
phosphate.
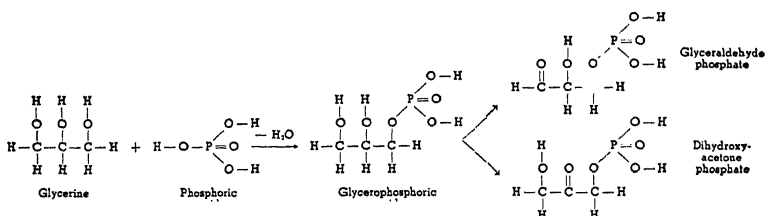
One of these molecules may now pass its hydrogen on to
the other. Such a dismutation, or oxidation of one molecule by
reduction of a fellow, is, of course, catalyzed by the proper
enzyme, a mutase. This results in the formation of phospho-
glyceric acid by the oxidation and of the familiar glycerophos-
phoric acid by the reduction.
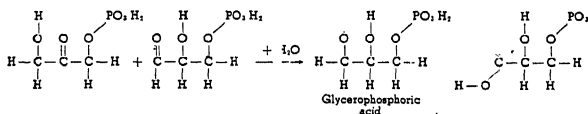
The phosphoglyceric acid finally splits off phosphoric acid
through at least two intermediate steps, leaving behind a mole-
cule of pyruvic acid.
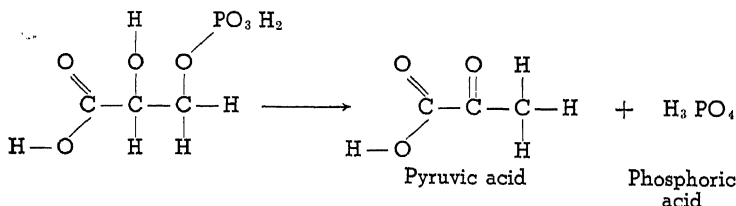
This is one of the paths of oxidation of glycerine, possibly not
the most important when plenty of oxygen is present. But it is

of particular interest since the pyruvic acid formed via this route is a chemical link between the fats and the sugars. In fact, as we shall soon see, when sugars are fermented the reactions involve practically these same substances all the way through.

Meanwhile what has happened to the fatty acids of the fat? These lose hydrogen atoms from alternate carbons so that molecular fragments containing two carbon atoms, such as acetic acid and glyoxal, tend to split off as the oxidation proceeds.
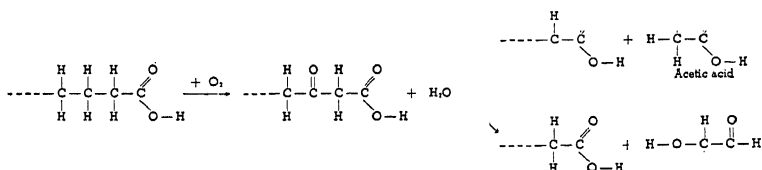
Such bits may be oxidized on to carbon dioxide and water; or they may combine in pairs to form, for example, acetoacetic acid which loses carbon dioxide in several steps of further oxidation and becomes acetone or pyruvic acid, our familiar landmark.
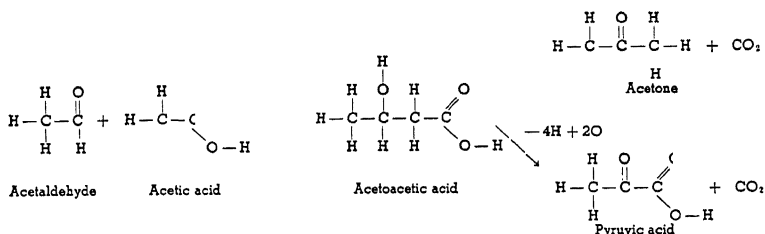
There is some reason to think that, as the fatty acids oxidize, besides the two-carbon units, some fragments with four carbon atoms split off. When the two ends of such a chain are oxidized the result, of course, is the familiar succinic acid. With so many points of contact between the chemical pathways followed within the cell by fats and carbohydrates, it is hardly surprising that one of these food substances is rather easily transformed into the other by the body. Eating starches makes

us fat; and from stored fat, glucose and glycogen can be produced.

The story of the proteins is, of course, somewhat more individual. The amino acids differ from the monosaccharides and fatty acids to a greater extent than they do from each other and, besides, each type of amino acid is a separate problem. The main difference, however, is in the presence of nitrogen in the amino groups and, once these are separated, the remainder of the molecule often treads the beaten path. The amino acid, alanine, for example, might form lactic acid if a deaminase split off its amino group.

Alanine   Lactic acid   Ammonia

Actually, in this case, such a simple schism does not occur, but deamination along with oxidation does. A joint loss of the NH_2 group and of two hydrogen atoms takes place with great ease, and the ubiquitous pyruvic acid is the result.

Alanine   Pyruvic acid

The more complex amino acids, especially those containing rings of atoms, require other special oxidizing enzymes to further their degradation. Tyrosine, for example, is attacked

Tyrosine

by tyrosinase and may form melanin, the blackish-brown pigment which colors our hair and skins and which is missing in

albinos. When acted upon by other enzymes other products result: in the cells of the adrenal glands, the hormone adrenal-

Adrenaline
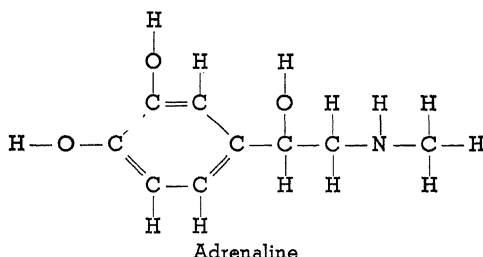
ine; in those of the thyroid (after combination with iodine and part of another tyrosine molecule), the hormone thyroxine.

Thyroxine
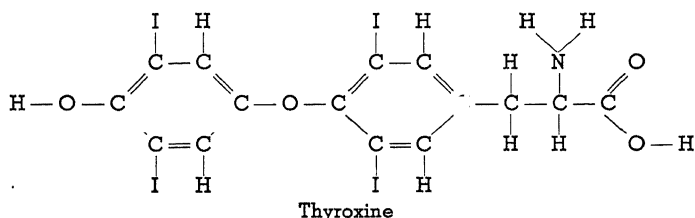
The detailed stories of the various amino acids are hardly our present concern, and for that matter are not yet too well known—this subject is now actively simmering in the heat of intensive research. But note again that in the course of the breakdown of proteins some of the same substances are formed as are produced by fats and sugars. It is not surprising, then, that the body can change protein into sugar or fat. The reverse, of course, is not possible unless sources of nitrogen are also available; but when they are, certain amino acids can be built by the animal body, and all the usual ones, of course, by plants. Even in the test tube, if oxygen is excluded and the needed enzymes present, pyruvic acid can combine with ammonia while reduced by succinic acid, and alanine be thus synthesized.

Chemical crossroads thus exist between the three major groups of food substances and protoplasmic constituents, not merely on paper but as reactions demonstrated to occur in cells or their extracts. It is more difficult to know what determines

the direction that any particular molecule will travel; why one
pyruvic acid molecule ends up as succinic acid, another as
lactic acid, a third as alanine, and others as carbon dioxide and
water. The presence or absence of oxygen, an excess or defi-
ciency of sugar or ammonia molecules, the acidity within the
cell and the concentration of particular cell ions, the activity of
special enzymes, and many similar factors collectively deter-
mine its fate. In vertebrates, particularly, still other substances,
the hormones, make their influence felt as well, often by
secondarily changing some of these primary conditions.

The thyroid hormone, for example, increases the concentra-
tion in the body cells of dehydrogenases, and to a lesser extent
of cytochrome oxidase, and so accelerates oxidations. One of
the several adrenal hormones aids in the formation of glucose-
phosphate, another influences the potassium and sodium con-
tent of the cell and probably the burning of sugar, while the
parathyroid hormone controls calcium. One of the many
pituitary hormones influences fat oxidations; and insulin, the
pancreatic hormone that prevents diabetes, helps to burn sugar
on the one hand and on the other prevents its formation from
proteins. Insulin, therefore, seems to block one of these chemi-
cal channels. A second pituitary hormone seems to keep it
open; at least, when the pituitary is removed as well as the
pancreas, the excess sugar production and decreased sugar
oxidation that follow removal of the pancreas alone largely
fail to occur.

Reactions Without Oxygen

Again we are getting ahead of ourselves and must return to
the metabolic web itself for a little longer. What are the chemi-
cal events which underlie fermentation? The problem here is
simpler than for respiration since only carbohydrates are con-
cerned in this and, of these, predominantly glucose. As in its
oxidation, glucose is first combined with one and then usually
a second phosphate molecule, and the hexose-diphosphate re-

sulting is split into two triose-phosphates, say glyceraldehyde-phosphate and dihydroxyacetone-phosphate, which we have already met in the oxidation of glycerine. These two substances dismute as before, to give glycerophosphoric acid and phosphoglyceric acid, and from the phosphoglyceric acid is formed pyruvic acid. The same coenzyme that is active in glucose oxidation enters likewise into these reactions of fermentation.

In most vertebrate cells, this is simply reduced to lactic acid, which is the end product of fermentation. True, the lactic acid may eventually be oxidized further under other conditions, or may even be rebuilt largely into glucose or glycogen; but these changes are not part of glycolysis. In the yeast cell, the pyruvic acid does not reduce directly to lactic, but is first decarboxylated to give acetaldehyde, which is then reduced to alcohol. Or, in the case of vinegar bacilli, the acetaldehyde is not reduced, but rather oxidized, so that acetic acid or vinegar results.
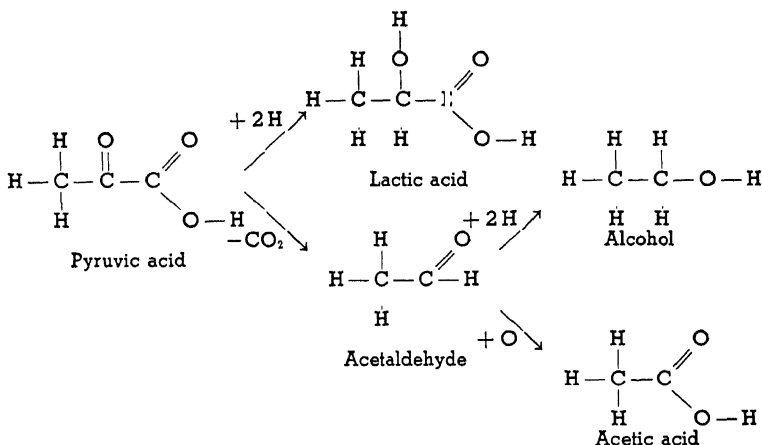
Still other branches in this chain of reactions exist at various levels, so that a wide array of particular end products of fermentation are formed by various kinds of cells. Indeed, a large number of these compounds—lactic acid, alcohol, acetic acid,

butyric acid, acetone, citric acid—are now produced by chemical industry in great quantities, with the aid of the proper microorganisms.

A few cell types, particularly those which man has deliberately cultivated to that end, depend on fermentation as their major source of energy and, even in the presence of adequate oxygen, ferment far more sugar than they burn. Others, and these include most if not all normal vertebrate cells, carry on fermentation only when oxygen lack or some interference with the oxidizing enzymes limits the respiration possible. Some cells possess both an ærobic glycolysis, carried on in the presence of air, and an anærobic glycolysis, proceeding in its absence. But in all cases the amount of fermentation occurring when oxygen is present is less than when it is absent, and an important quantitative relationship exists between respiration and fermentation.

It can be stated roughly as follows: every molecule of sugar which is burned prevents the fermentation of three (to six) molecules of sugar. Note that it is not only the amount of oxidation that is important, but more particularly the amount of sugar
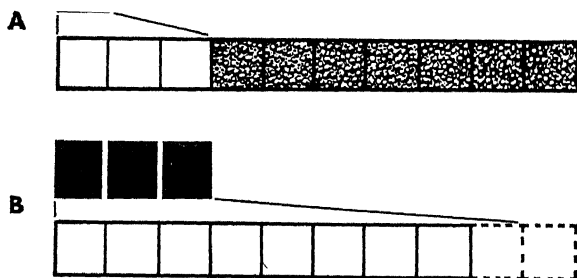
FIG. 77. A, brewers' yeast; B, wild yeast. Solid squares, above, show amount of glucose burned in oxygen; open squares, below, amount fermented in lack of oxygen. No fermentation occurs in the presence of oxygen in wild yeast, for the Pasteur reaction suffices to prevent this; but in brewer's yeast ærobic fermentation does occur (stippled squares). The lesser oxidation of glucose in this yeast does not suffice to stop the greater anærobic fermentation.

oxidation. Brewers' yeast, for example, burns ten arbitrary units of glucose per hour when oxygen is present. When oxygen is absent it ferments one hundred units in the same time. If the above relation (known, by the way, as the Pasteur reaction, since his genius first clearly recognized that "fermentation is life without oxygen") holds, then in the presence of oxygen there should still be seventy units of glucose fermented, since the oxidation of ten would prevent the fermentation of only thirty; and this is the case. With wild yeast, which burns thirty units of sugar per hour in oxygen and ferments eighty, let us say, in its absence, no aerobic glycolysis occurs, for the burning of thirty would prevent the glycolysis of ninety, more than sufficient entirely to suppress the whole anaerobic fermentation.

How this actually works in the cell is not yet clear, though important hints are available. If, for example, glucose must pass through pyruvic acid on its way both to carbon dioxide and to lactic acid, then clearly the fate of the pyruvic acid molecules could determine the course of the whole reaction. When more of these were oxidized, fewer would end as lactic acid; when none were oxidized, all would end as the acid. If the glycolytic enzyme becomes more active when it is reduced, which we have seen is probable, a mere decrease in oxygen in the cell could start or hasten the formation of lactic acid even though the usual amount of pyruvic acid continued to be burned. In some cases just this occurs.

It has been possible, by subjecting cells to various injuries or by adding particular enzyme poisons, to show the intimate relation between the oxidation and the fermentation of glucose and to prevent one or the other or both of these processes. A fairly simple organic substance, iodoacetic acid, prevents the dismutation and breakdown of the triose-phosphates, and so the formation of lactic acid (or alcohol) and the oxidation of sugar in cells. If, however, those substances whose formation was prevented by the poison are added to the cell, they continue the

chain of reactions in normal fashion. Pyruvic acid, for example, can still be reduced to lactic acid anaerobically and it or lactic acid can be oxidized when oxygen is available. Certain other poisons block only sugar oxidation, so that glycolysis continues at its maximum rate although oxygen is present; or, conversely, block glycolysis, so that, although sugar is burned normally in oxygen, fermentation does not set in in its absence. Finally, certain agents, or for that matter even high temperatures, are able to break down completely the tie-up between the two processes, so that the Pasteur reaction no longer is manifested and fermentation and respiration occur quite independently of each other.

The complete separation of these major metabolic processes under such conditions suggests that some special mechanism, besides their having pyruvic acid as a common step, normally is necessary to connect them. It has, indeed, been believed that a specific enzyme, the Pasteur enzyme, mediates this interchange, perhaps by controlling the oxidation of -SH groups in a glycolytic enzyme, and that it is the inactivation of this which keeps the Pasteur reaction in abeyance. Whether or not this be so, it remains true that the intimate relation between oxidation and glycolysis is very general and very important, and when it is interfered with striking consequences may result. Part of the chemical pathology of the cancer cell, for example, is the loss of the Pasteur reaction, so that even when plenty of oxygen is supplied, a cancer continues to produce lactic acid in large quantities.

So far we have watched especially the changes in the hydrogen and carbon atoms of molecules. In oxidations and, less directly, in fermentations, hydrogen atoms are given off from one molecule, a hydrogen donator, to another, a hydrogen carrier, which temporarily combines with them but which soon passes them on to another carrier, until finally they become attached permanently to the hydrogen acceptor. In nearly all cases it has appeared that the phosphate group is concerned in

these reactions. Glucose does not split to two triose molecules until after it has combined with phosphate, and these, after undergoing their interactions, must free themselves of this attachment before completing their final changes. There is, in fact, a complicated phosphate cycle in the cell, of no less interest than the hydrogen sequence.

Glucose does not combine with inorganic phosphate ions but takes on the phosphate group from particular phosphate donors. The triose-phosphates, in turn, do not simply split off this group, but ordinarily pass it on to form a new combination with a phosphate acceptor. Several cell substances are now known which help complete the phosphate cycle by alternately taking on and yielding up the phosphate group. One of these is creatine, which, under the action of the appropriate enzyme, of course, forms creatine-phosphate. The creatine-phosphate can easily pass on phosphate to another compound. More important is a group of substances which, though they do occur unattached in the cell, are most commonly combined to form nucleic acids, and which are therefore known as nucleotides. Adenylic acid, one of these, can combine with one or, most commonly, two phosphate groups to form adenyl-pyrophosphate, which normally donates phosphate to the glucose molecule.

Phosphates are certainly essential to the fermentation of sugar by yeast and by muscle and, interestingly enough, this very adenyl-pyrophosphate, which directly enters into the glycolysis reactions, is as well a coenzyme for one of the glycolytic enzymes. This is certainly a fancy means of pulling oneself up by the bootstraps. The more adenyl-pyrophosphate there is, the more rapidly can hexose-diphosphate be formed for subsequent glycolysis; and at the same time, the more rapidly will certain stages in this glycolysis go forward. On the other hand, this dual role affords an adroit means of control. With a limited amount of phosphate present, the more of it that is donated to the glucose molecule, the less remains to

form adenyl-pyrophosphate; and the increased glycolysis so
made possible at an early stage is compensated by a decreased
rate of the later reaction which requires the coenzyme. It is
perhaps clear by now how intimately these many processes
play into one another's hands and how a slight change in the
conditions within a cell may suffice to direct important reac-
tions now one way, now another.

Regulation

The amazing thing, of course, is that, despite all the enticing
byways, the metabolism of cells marches forward so steadily
and consistently along its chosen route. Second after second
and day after day the rate at which oxygen is used by any type
of cell is constant while the cell is at rest. During special activ-
ity the rate may increase many fold, closely paralleling in
quantity the amount of work to be done; yet after some minutes
the respiration falls back again quite smoothly to its original
resting rate. Each kind of cell has, of course, its own charac-
teristic tempo, different from that of most others, to which it
tenaciously clings or returns. Such regularity, quantitative even
more than qualitative, bespeaks an accurate control; just as
the constant temperature of our bodies or of our homes depends
upon the presence of regulators.

For one thing, of course, the temperature of the cell controls
the chemical rate, since metabolic processes in cells, like
chemical reactions in test tubes, depend on molecular move-
ment and so on temperature. Indeed, the cold-blooded animals
are seriously handicapped by the caprices of weather; it is no
trick to pick a fly off a cold window pane in the fall but quite
another matter to catch it on a hot summer afternoon. The
speed of its chemical reactions, and therefore of its muscular
ones, goes up and down with the temperature. Possibly the
final ascendancy of mammals over the huge reptilian dinosaurs
of an earlier geologic epoch was due more to their having

acquired the ability to keep up body temperature in the cold, or warm-bloodedness, than it was to the increased size of their brain and the enhancement of their intelligence.

In the warm-blooded animals, and in the great hordes of organisms living below the surface waters of the ocean, the temperature is very constant; this makes possible, though it does not alone insure, a constant rate of metabolism. Other factors acting upon cells, especially in complex organisms, can also modify their basic tempo. When a cell is directly stimulated to activity, in single-celled organisms by some chemical or physical change in its environment, in the multicellular ones by chemical messengers reaching it in the circulating fluid or by nerve impulses racing along special private wires from the nervous system, its metabolism is temporarily accelerated. This process of stimulation must be examined later in detail, but we have already seen how certain hormones acting upon the cell may modify fairly permanently its chemical speed. The same is true also of various vitamins, such as ascorbic acid, which directly participate in the reactions of the cell. The important role of salts has already been indicated.

Yet we are still really begging the question of how the chemical exchange in the cell continues with such regularity. Ordinarily when substances are mixed together and react, the change starts off at a high rate, falls off rapidly at first and then more slowly, and finally stops when the reaction is complete. Only if sufficient supplies of the reacting substances are continuously added will the speed be maintained, and even then, as the products of the interchange accumulate, they rapidly swamp out the reaction unless they are as continuously removed. A fundamental law of chemistry states that, if two substances, A and B, react to form two others, C and D (and these, of course, in a reversible reaction, react to form A and B again), the rate of the forward change increases in proportion to the concentration of A or of B and is therefore given by the product of their concentrations. The rate of the backward

reaction is similarly given by multiplying the concentrations of C and D. When change is proceeding with equal speed in both directions, at some particular set of concentrations of the four substances, then the system is in equilibrium and their amounts remain constant.

Thus succinic acid and oxidized cytochrome react to form fumaric acid and reduced cytochrome, and vice versa. Starting with the first two alone, the succinic acid will oxidize, at first rapidly, then ever more slowly, until an equilibrium mixture of all four substances is formed. Of course, if the reduced cytochrome produced is continuously reoxidized by something else, and if fresh succinic acid is as steadily supplied from other sources, then the reaction will go continuously forward to fumaric. Note also that the initial speed of oxidation should be more rapid when the amount of succinic acid present is increased. Such relations are obeyed by isolated enzyme systems and their reactants in the test tube.

In the cell, this is rarely true. The rate at which oxygen is consumed can be decreased, it is true, by decreasing the concentration of oxygen available to the cell; but most cells are unaffected as oxygen concentration is steadily lowered until it is almost all removed. Conversely, when 100 per cent oxygen is supplied to the cell in place of air with its 20 per cent, respiration is not increased at all. Yet oxygen is one of the basic reactants in cell oxidations. The same is true for foodstuffs; if these are entirely withheld the respiratory rate may fall precipitously; but whether a little is present or a great deal makes slight difference indeed. Likewise for the end products of metabolism. Water, of course, is so plentiful in all cells that its concentration is not changed significantly by the additional molecules produced from oxidations. Carbon dioxide concentration can vary over a considerable range within the bounds of normal with no effect on respiration, though it may slow cell processes when increased beyond these bounds.

No, some condition other than the concentrations of reacting

molecules ordinarily must set the rate; why not the activity of those enzymes which make the reactions possible in the first place? So long as sufficient reactants are available to keep the enzymes working to capacity, additional substrate molecules must simply wait their turn without influencing the exchange. When more enzyme molecules or ones which carry out the change more rapidly are present, the rate will be increased. Nothing else can hasten metabolism. Consider an elevator carrying people to the upper floors of a building. With few individuals about, the number of people alone determines how many reach a given floor; but as a crowd gathers, so that the elevator is full each trip, only the speed with which the trip is made and the number of elevators running limit the traffic.

A serious problem now confronts us: if at rest the cell enzymes are working to capacity, how can the metabolic rate be suddenly increased, often ten times or more, when the cell is stimulated to activity? The answer is complicated and incomplete, but follows two main lines. For one thing, more active enzymes must participate. This could hardly depend on the sudden building up in the active cell of complete new molecules of these complicated stuffs, but it could easily involve a slight chemical change in these molecules. As we have seen, a glycolytic enzyme is active only when its sulphur atom is reduced. A single and very easy change from the -SH to the -S-S- form would throw out of action the entire molecule. So large reserves of inactive enzyme molecules or proenzymes might become activated as a result of stimulation of the cell. Such proenzymes are indeed widely known. Even the powerful trypsin in the digestive juice from the pancreas is unable to exert its splitting action on protein until the trypsinogen, its proenzyme in the pancreatic juice, is activated by another substance formed in the intestine.

Or the enzyme might itself remain unchanged while other adjuvant substances, much smaller and simpler molecules, were increased in concentration. Thus a sudden formation of

adenyl-pyrophosphate, to serve as coenzyme, could hasten glycolysis; and this, in turn, might be produced by the simple accumulation of phosphate ions. Or, finally, other substances in the cell might hold the enzyme in check and their removal would release its full power. Such inhibitors are also known. Pyrophosphate itself, for example, strongly suppresses iron catalysts; and the combination of pyrophosphate with adenylic acid might thus remove an inhibitor as well as form an accelerator. In terms of the elevator analogy, the traffic carried could be increased by opening up new units, by using stronger motors to increase the speed of those in use, or, if there were such, by loosening their brakes.

Let us extend the analogy still further. Suppose a barrier with a narrow passage admits people to the elevators one at a time. The rate of transportation, though again constant, does not now depend upon the capacity of the elevators or on the size of the crowd outside, but only on the number of people gaining entrance. But if the gates are suddenly swung wide, the crowd will move at a new, far more rapid, rate determined only by the elevator facilities. So also in the cell. It is probable that substrate normally is not given free access to the enzymes but is partially separated from them, adsorbed on or imprisoned behind various cell surfaces. The resting metabolic rate might then depend only on the steady dribble through these barriers of a regular file of substrate molecules; the increase on activity, to an opening of wider passages.

Just such an increase in membrane permeability does characteristically accompany the stimulation of cells. This permits the substrate molecules to flood down upon those of the enzyme, which whirl through their paces as fast as they are able. In liver cells, for example, there are simultaneously present glycogen molecules and the enzyme that can vigorously hydrolyze them to glucose. Under slightly changed conditions —lack of oxygen, presence of certain hormones, even moderate narcosis—the hydrolysis occurs almost explosively and the

glycogen completely disappears. Since narcotics act by coating surfaces, and in so doing often displace from a surface the previously adsorbed molecules, it is tempting to interpret such facts in terms of the sudden release of glycogen from a position in the cell which protected it from the enzyme molecules, and a rapid reaction when they finally meet in the protoplasmic matrix.

Another example is presented by the sea-urchin egg, which increases its rate of respiration some sixfold when fertilized. There is no question that structural changes occur promptly after the sperm enters, for a visible disorganization of the surface can be watched under the microscope as it travels, during half a minute, from the point of entry to the opposite pole. And associated with this superficial alteration are similar though less overt changes in the deeper

FIG. 78. Microphotographs of living sea-urchin eggs. A, not yet mature. B, mature. C, act of fertilization by a sperm (three are clearly visible). D, fertilization membrane starting to rise at region of sperm entrance. The great mass of sperms now present is held away by the membrane. E, membrane fully developed. Fertilized egg will begin division in a short time. (Photographs by courtesy of Ethel Browne Harvey.)
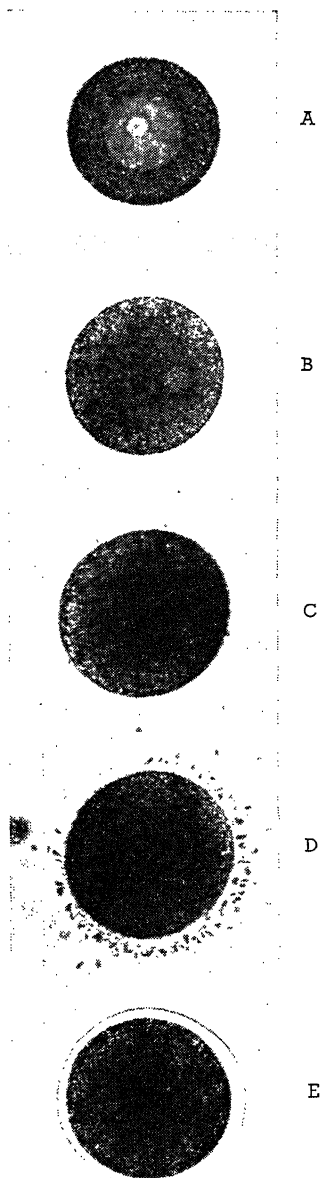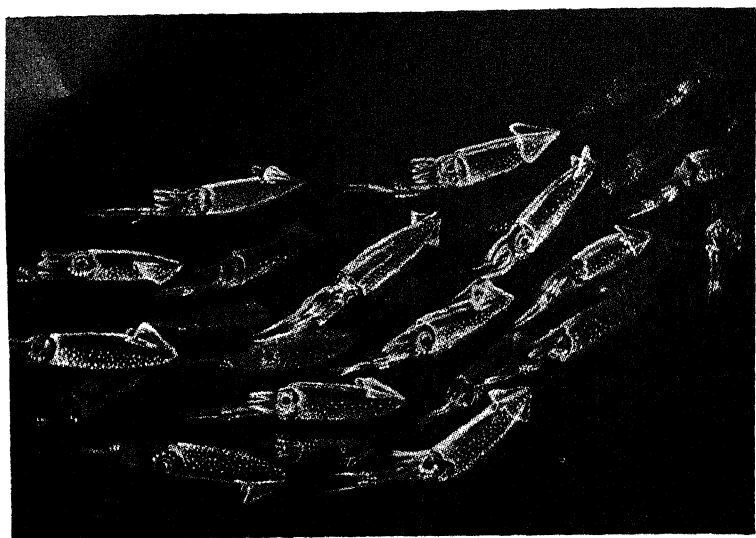
FIG. 79. Squid seen in the light of their own luminescence. (From Dahlgren, by courtesy of The Franklin Institute.)

cytoplasm which release adsorbed cytochrome molecules. Their non-availability is the factor that keeps the resting respiration in check and their sudden liberation is responsible for its increase after fertilization.

Still another case is that of animals which can glow or luminesce. Many of these depend for their light on the oxidation of a substance, luciferin, which reaction can take place in the presence of oxygen under the control of an enzyme. Substrate, oxygen, and catalyst are all present together in the resting cells and nothing happens. But let the animal be disturbed and send nerve messages to excite the light-producing cells, and the barrier goes down. There results a chemical explosion, a flash of light, and oxidized luciferin.

Thus, we recognize again the profound importance, for maintaining the proper kind and amount of metabolism, of the spe-

cial substances present in protoplasm and of their peculiar organization.

The Case of Muscle

Let us examine, in terms of the above, the metabolism of a muscle cell at rest and, especially, at work. Probably no other living entity has been subjected to such intensive chemical study as has the striated cell of a leg muscle of the frog; and a glance at what it has taught and how we have learned it is worth while. One need only recall the long period of deep and rapid breathing which follows even a short intense muscular effort to be convinced that muscles contracting use more oxygen than they do at rest. This can be studied more directly on a single isolated muscle either left quiet or stimulated, by electrical currents or through its nerve, to give maximal contractions. The rate of oxygen consumption at rest is increased over ten times during activity; and, as might be expected from the after-effects of exercise, this increased respiration tapers off very slowly to the original resting level during a half-hour. The more work the muscle does on contracting, the more oxygen it uses; and it is clear enough that this work ultimately depends on oxidations.

Yet such a muscle kept in an atmosphere of nitrogen not only survives for many hours but gives perfectly normal full-sized contractions for a considerable time. It was long supposed that this was possible because the muscle during "good times" stored up in loose chemical combination a supply of oxygen upon which it could draw when the outside supply failed. Such an oxygen reserve would, to be sure, have been extremely useful biologically; but perhaps this was too difficult a chemical problem for even protoplasm to solve, or maybe another kind of solution was more satisfactory. In any event,

there is no significant oxygen reserve in muscle; and during its stay in nitrogen the muscle produces little or no carbon dioxide, as it should if oxidations were continuing. (A small amount of myoglobin in muscle, related to hemoglobin in blood, does hold enough oxygen for a contraction or two, but this is of no consequence to a muscle giving hundreds of twitches in nitrogen. It may be of importance in normal activity.)

Another discovery helped explain this apparent contradiction. Muscles resting in the absence of oxygen slowly become acid, and when active they acidify more rapidly. Only thirty years ago, by laborious chemical analysis, was it shown that this acidity is due to the accumulation of lactic acid, slowly when at rest, rapidly during contraction. When oxygen is later readmitted, the lactic acid slowly disappears and the muscle takes up a much greater amount of oxygen than it would use at rest. An "oxygen debt" which the muscle had built up while "asphyxiated" is now discharged, and the lactic acid that had accumulated disappears. It seemed, then, a reasonable hypothesis that: during rest, and still more during activity, carbohydrate is changed to lactic acid—and it was soon shown that glycogen decreases as lactic acid forms—and the lactic acid, in turn, is promptly oxidized to carbon dioxide and water. If oxygen is lacking, the second step is, of course, prevented, and the lactic acid accumulates until changed conditions permit its subsequent oxidation.

Now, it is easy to calculate the amount of oxygen required to oxidize a given amount of lactic acid to carbon dioxide and water, and to compare this with the extra oxygen actually used by a muscle which is removing accumulated lactic acid. Only about one-fifth as much excess oxygen is used as would be needed to oxidize the lactic acid which disappears. Clearly, not all the acid could have been completely burned. Perhaps all the lactic acid molecules are only partly oxidized, say to pyruvic acid or to acetic acid, instead of all the way to carbon dioxide and water. Yet the amount of carbon dioxide produced,

compared with the oxygen used, shows that oxidations were complete. One-fifth of the lactic acid must have been entirely burned—what had happened to the remainder? Again careful analysis of the muscle sugars shows that, as lactic acid disappears, glycogen increases and, indeed, by just the amount required to account for the four-fifths of the lactic acid not burned.

Here, then, is a satisfying picture of the chemical machinery of muscle contraction. During the actual shortening, when work is done, large quantities of glycogen are changed to lactic acid, with the liberation, of course, of a certain amount of energy. Later, during the recovery process, most of the lactic acid is reformed into glycogen, the chemical machine is reset, and only a small part of the lactic acid is burned with oxygen to supply the energy required for this rebuilding. The contraction itself is perhaps caused in some way by the sudden production of acid within the muscle cell; for glycogen is neutral and lactic acid, as its name suggests, is an acid, in fact a rather strong one.

This scheme, simple though it seems, took twenty years in the building and the combined efforts of dozens of investigators in laboratories all over the world. Large numbers of facts had been ascertained and, though an occasional irregularity was hard to account for, the great mass of them fitted excellently and in detail into this picture. And then, just over a decade ago, a scentific bombshell burst—muscles were found to contract without producing lactic acid! As so often happens, several discoveries were made nearly simultaneously, all indicating the same thing. Most important was that iodoacetic acid (which we have seen prevents the formation of lactic acid, though this was not known when these experiments were begun) can poison muscles so that they do not form lactic acid, to be sure, but they still contract. True, the poisoned muscle could not continue for a long time—after a few hundred twitches it remained in a contracted state, or rigor; still it had done a pretty good job with no help from lactic acid and with-

out developing any acidity within the cell. What, then, was the energy source for this contraction and, in the absence of acid, what was the chemical machinery for producing the shortening?

Fortunately, just before these experiments were performed, a new substance had been discovered, and in muscle—creatine-phosphate. It was soon shown that in these poisoned muscles this compound broke down to creatine and phosphate during contraction, so here was a possible source of energy for the work. Further, since creatine-phosphate is a fairly strong acid and its breakdown products are much weaker ones, this change led, not to acidification, but rather to an alkalinization of the muscle cell. Theory, particularly in its early stages, is often very labile. If the actual shortening of a muscle cell did not depend on its becoming acid, why then it was just as satisfactory a theory to have contraction depend on the cell becoming alkaline. In a somewhat more sophisticated form, such is the present view. At least we are now certain, by direct measurement of the change, that a muscle does become less acid during contraction, and we have a pretty good picture of how this shortens the cell—but that comes later.

To return to the chemical sequence, the picture has not changed so completely as it might seem. Instead of glycogen breaking to lactic acid during contraction, we now have creatine-phosphate breaking to creatine and phosphoric acid. But this reaction is promptly reversed and the creatine-phosphate rebuilt, which requires energy. This is supplied by the breakdown of glycogen to lactic acid, which follows close behind the first reaction. Finally, as before, the cycle is completed with the aid of oxygen, which burns some of the lactic acid and gives energy to turn the remainder again into glycogen. Note, however, that the lactic acid formation is no longer considered responsible for the contraction itself, but is regarded rather as one of the steps in the recovery process; and improved methods and more careful analyses have shown, in fact, that the muscle

lactic acid increases not during but immediately after the actual contraction. Further, the question may be raised: why must some of the lactic acid be oxidized to give the energy needed to restore the remainder to glycogen; could not the burning of any food substance by oxygen equally well supply this energy? Here again more recent experimentation has shown this to be possible. We have now a series of three reactions: during contraction, the creatine-phosphate change; during recovery, first, the formation of lactic acid and, finally, increased oxidations.

Surely we should now be satisfied. Unfortunately, nature is what it is, and man in his fumbling manner can only approach closer and closer to discovering it. Adenyl-pyrophosphate was discovered soon after the experiments just described. It also breaks down rapidly to adenylic acid and phosphate when a muscle contracts and is reformed afterward. In enzyme-containing muscle extracts, creatine-phosphate added to adenylic acid rapidly transfers its phosphate, so that adenyl-pyrophosphate is produced. Possibly, then, here is an even earlier step in the reaction chain of contraction. Adenyl-pyrophosphate might yield the immediate energy for contraction, and creatine-phosphate breakdown and restoration, like that of its predecessor in the limelight, lactic acid, be relegated to the recovery process.

Adenyl-phosphate →Adenylic acid + Phosphate + Energy
Creatine-Phosphate + Adenylic acid →Adenyl-phosphate + Creatine
Glycogen + Creatine + Phosphate → Creatine-phosphate + Lactic acid
Oxygen + Lactic acid → Glycogen (4/5th) + Carbon dioxide + Water

The evidence here is not quite so decisive as in the case of lactic acid, and it may even turn out that adenyl-pyrophosphate or creatine-phosphate may either one break down first, depending on conditions, to be restored by energy from the breakdown of the other. Normally, however, the nucleotide seems to precede. (Incidentally, the breakdown of the adenylic compound, like that of the creatine one, leads to an increased alkalinity

in the cell.) It is also possible, of course, that even adenyl-pyrophosphate does not supply the initial energy for contraction. At any moment still another substance might be discovered and placed at the head of the line. At least, previous experience urges caution before concluding that now we know the whole truth.

Fortunately, even these possibilities can be indirectly explored. When glycogen breaks to lactic acid, or either of the phosphate compounds splits to its constituents, the total number of separate molecules or ions is increased. Now certain properties of a solution, as osmotic pressure, depend only on the total number of dissolved particles, irrespective of their size, so that such a series of chemical breakdowns should increase the osmotic pressure of the muscle cell. Conversely, if a change in osmotic pressure (or, its more easily measured equivalent, the vapor pressure of the cell's water) can be measured, the number of new particles formed can be calculated. The calculated chemical changes could then be compared with the total of known changes of the various substances that participate in contraction and recovery.

Such a measurement would, of course, be very difficult to make during a muscle twitch, not only technically but theoretically, for one reaction is always being reversed as the following one proceeds. Under conditions of continued, regular stimulation, however, particularly if the cells are kept asphyxiated, the various splittings can run to completion, for their restoration finally depends on oxygen. It is possible, then, to measure the osmotic change and to equate it with the total measured chemical changes. If the increased number of particles already accounted for proves equal to the total increase shown osmotically, then no other still-unknown reactions remain. Such experiments have been made, and that is the result.

There is, then, some basis for believing that at last we have reached the end of the chain, despite the loopholes that remain.

Even if this be entirely correct, however, it may still be true, as indeed some evidence already suggests, that adenyl-pyro-phosphate breakdown is, after all, the first step of the recovery process and that no chemical breakdown at all accompanies contraction itself. If energy is stored in some physical or physico-chemical form—as in a compressed spring, or a charged condenser or battery, or a raised tank full of water—then a simple release of this energy—by pulling a catch, completing a circuit, or opening a valve—is all that is required. The energy from chemical reactions would then be needed to reset the physical machine, but this would be part of the recovery rather than the contractile process.

But here we are once again sharply against the problems of excitation itself and of what physical mechanisms and trigger releases may exist in cells, which must be deferred for later discussion. More immediately, the question of work and energy has willy-nilly crept into our chemical considerations as soon as they were applied to the functioning of an actual cell; and the problem of energy now merits further attention.
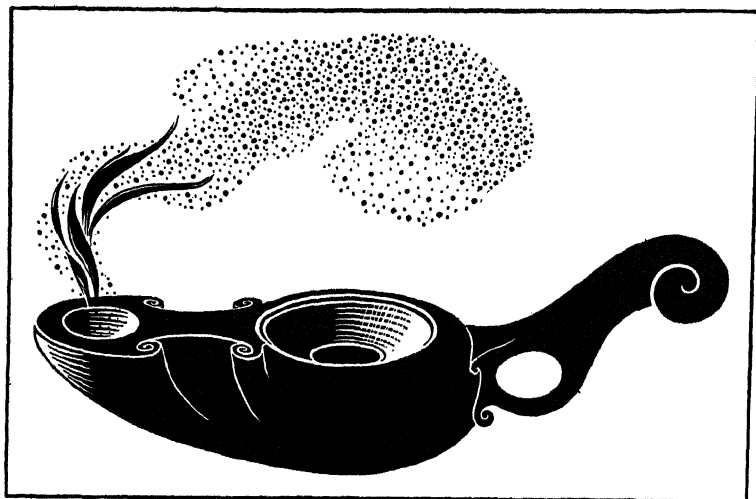
Chapter Seven: Fuel for the Lamp of Life—Energy

Life marches on! Into the molecular whirlpool that is the cell come forever foodstuffs, and from it leave wastes. When the chemical hurly-burly is stilled, life stops. But why should this be? The great bulk of molecules that enter the cell, under certain conditions possibly all of them, are simply ripped apart and their fragments spewed out again. A machine can be stopped and started again—why is not the cell equally able to perform its chemical reacting or to remain quiescent; and for that matter, why should it perform this uninspiring task at all? Why is the considerable and continuous catabolism useful and necessary?

The answer, of course, is "energy." When sugar is burned to carbon dioxide and water, when glycogen splits to lactic acid, when creatine-phosphate is hydrolyzed to creatine and the phosphate ion, energy is released. It is not difficult to see that this chemical energy is necessary for a working cell. The muscle does mechanical work in contracting, the gland performs osmotic work when it secretes substances from more dilute to more concentrated solutions. Other active cells pro-

duce electric currents or flashes of light or change their form and structure or wave their cilia. In all cases energy is required, during both action and the subsequent recovery and resynthesis. But what of the cell at rest, not moving, not secreting, not even growing or dividing—for growth means new structures and more surface energy stored? Does the quiescent cell also need energy, and if so, why?

Certainly the continued metabolism of the resting cell is essential, for when it is interfered with the cell dies. One normally using oxygen may survive some minutes or even hours in its absence, but ultimately succumbs. The chemical products of these oxidations are clearly useless to the cell, since it promptly excretes them; it must be the energy that is required. (Some of the intermediates are, of course, a part of active protoplasm and are needed to keep the chemical chain unbroken— as heat is needed to keep the autocatalytic candle flame alight.) That this is so is shown further by the chemical events that occur when oxygen is withheld, for then fermentation promptly sets in and sugar is broken down at a greater rate than when it is oxidized.

A gram of sugar burned to carbon dioxide gives 3,800 calories, whereas when it is split only to lactic acid it gives but 380. If the cell splits sugar only for the energy it obtains, and not because of any utility in removing glucose or in forming carbon dioxide or lactic acid or any other particular substance, then for some given amount of energy it must ferment ten times as many molecules as it would have to oxidize. But this is just the sort of relationship already seen: for each molecule of sugar which fails to be burned three to six molecules are fermented, and phosphate compounds split as well. The whole procedure makes sense if, and only if, the cell's concern is primarily with the energy obtained from these reactions and not with their chemical by-products.

But still, why does the resting cell need energy? For the same reason that the whirlpool does, to maintain its organization. A sound motor boat floating on a lake uses no energy at
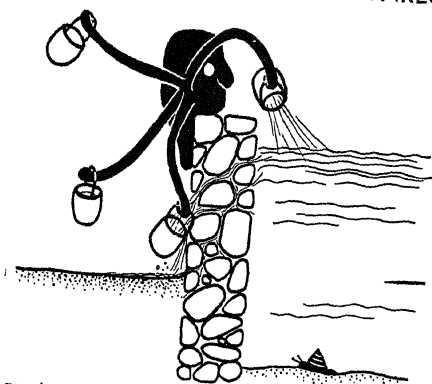
Fig. 80. Cells must continuously expend energy to do work in maintaining the integrity of their membranes and to counter the steady leakage of water and dissolved substances through these semi-permeable "dikes."

rest, though considerable amounts are needed when it is at work and going somewhere. But if it leaks, it will slowly fill and sink if no energy be expended to keep it afloat. A small pump running continuously is needed to keep the bilge from rising. A feeble but steady expenditure of energy keeps the system intact, ready at any time to do something useful. "Going places" requires a greater energy expenditure, but the maintained dribble is vital to merely staying afloat. The same is true of cells. Their semi-permeable membranes leak, they tend to depolarize and lose their charges; and such discharged cells are "sunk" if the condition lasts for any time. To maintain their functional integrity, finally even their structural one, cells must have a continuous supply of energy.

Is it not a sign of poor craftsmanship that cells have such leaky membranes and so fragile an organization that they are perpetually disintegrating of themselves? No, this is rather the necessary price paid for flexibility and control. A suspended bar magnet may hold up a piece of iron indefinitely without any use of energy. Gravity pulls, but the magnet pulls harder and the iron remains hung in space. But it is permanently fixed, unless dislodged by a sufficiently strong force from the

outside. Not so with the electromagnet, the iron core of which retains its magnetism only so long as current is flowing in the wires coiled about it. Its pull varies in strength with the amount of current and depends on a continued supply of energy. Yet the electromagnet, despite the obviously greater cost of operating it, is the type used overwhelmingly in industry and in the laboratory. It possesses the invaluable attribute of pliability and control. The steel ball can be held or dropped or raised again; other magnetic fields can be generated; currents made to flow or stop. The homely buzzer, the telephone, the generator, innumerable other members of the family of appliances on which civilization is built, are made possible by the modulation of electromagnetism. In the cell the same principle applies. If its membranes, for example, were rigidly and safely impermeable at rest, activity, which involves their temporary breakdown or alteration, would be more difficult or impossible.

So much then is certain: the cell machinery, like any other kind, needs energy to make it run and do its work. In addition, unlike most man-made instruments, it requires energy to maintain its delicately adjusted status quo. What is still far from clear is how this energy is captured by cell mechanisms and routed to its useful ends; for liberated energy, unless it flows through some organized system, is simply dissipated as heat. Gasoline poured on the ground and lighted only warms its surroundings; the same burning in the cylinders of an automobile yields part of its energy as mechanical work in moving the car and its parts. Only secondarily, through friction, does this degenerate to heat. Rain, falling on a roof and dropping off the edges, slightly warms the ground it hits, or at most shifts around the dirt particles to dig unpleasant little holes in the garden. The same water, discharging the same potential energy in falling from roof to ground, if led down a rain-spout, can turn a small water wheel which will contribute its bit of useful work in any way desired.

The organized machinery of the cell is part of its very struc-
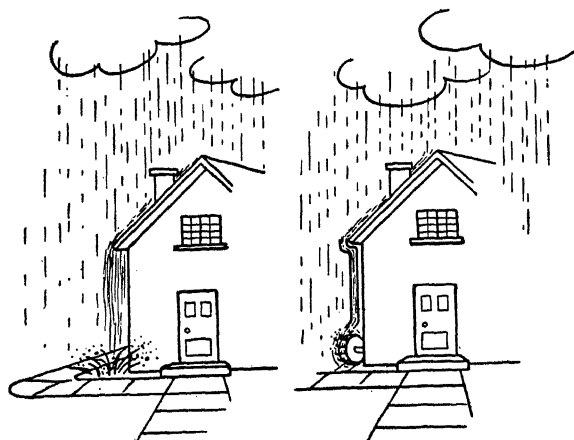
FIG. 81. Chemical energy released when molecules react, like the energy released when water falls from a roof, can do useful work only when it is made to pass through some organized machinery. When it is simply freed at random it is all dissipated as heat.

ture, and it is not surprising to find that, not only is energy important in maintaining surfaces and membranes, but, to be useful, it must be released on or in relation to them; it is not surprising, indeed, that the cell's oxidizing catalysts utilize surface reactions so extensively. It is simple to demonstrate the necessity of delivering newly liberated chemical energy directly into the hopper if it is to be useful. But before examining this evidence, we must digress a way into other considerations.

Energy Sources

Forget again the complex series of intermediate reactions that occurs when a substrate, say sugar, is oxidized, in favor of the simple summary reaction:

$$\text{Sugar} + \text{oxygen} \longleftarrow CO_2 + H_2O + \text{energy.}$$

Does the energy come from the sugar, from the oxygen, or, in some way, only from the interaction between them? The

answer is rather surprising, since it turns out that the sugar, far from giving up energy when it is oxidized, actually requires the addition of some. Negative electrons must be pulled away from their positive nuclei when carbon atoms in the sugar molecule are oxidized and, just as does pulling apart oppositely charged magnets, this takes work. When some atom of another molecule, in turn, is reduced, these free electrons drop into its sphere of attraction, their potential energy is released, and work can be done. The total energy freed, then, is the amount produced when the oxygen atoms are reduced minus the quantity used up as the carbon atoms are oxidized. Just what the balance is in each case depends on the total structure of the molecule, for electrons are not held equally firmly by all carbon atoms in their different combinations. The first pair of electrons can be pulled from carbon with less energy than can the next pair, so that methane is oxidized to methyl alcohol with greater ease, in terms of energy, than is the alcohol further oxidized to the aldehyde.

Methane   Methyl alcohol
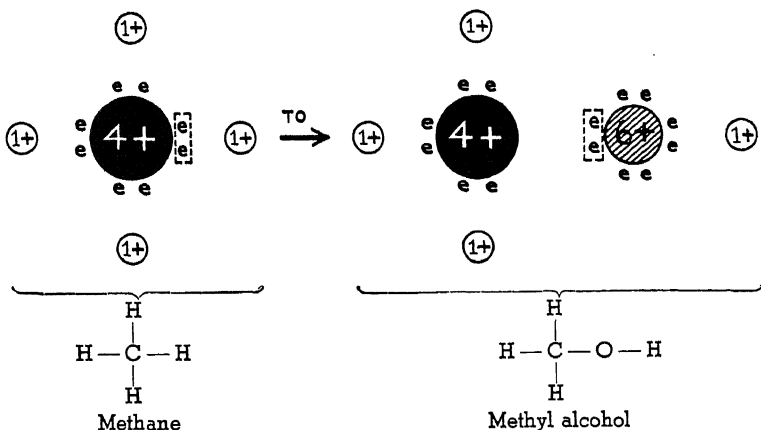
The total energy of the reaction will depend, of course, no less on the atoms which are reduced than on those oxidized. When oxygen is the oxidizer and takes up electrons, large

amounts of energy are released. When one carbon atom is reduced while another is oxidized, the two energies, of pulling out and of dropping in electrons, are much more nearly equal, as one would expect, and the free energy left over in the balance is far less. This is easily shown, for example, in the oxidation of an aldehyde to an acid. Formaldehyde plus oxygen gives formic acid plus fifty-eight units of energy (calories). If the aldehyde molecule, however, is oxidized by another identical aldehyde molecule, so that a carbon in one loses electrons and a carbon in the other gains them—as occurs when the aldehyde dismutes to acid and alcohol, as in fermentations— the total energy freed is only four calories.
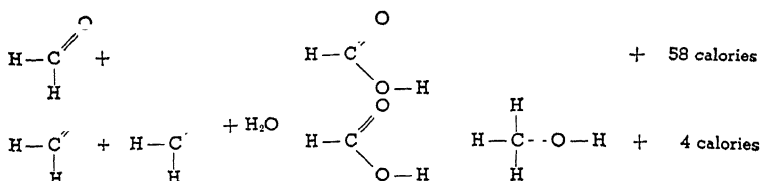
So universally is this quantitative difference true that it can be said: cells obtain their energy overwhelmingly from oxidations by oxygen. Oxidations by other atoms, in whatever molecular form, and particularly those by carbon atoms, yield unimportant amounts of energy. We shall see, however, that they are of the utmost importance in terms of the chemical substances so produced, for it is commonly by the reduction of such oxidizing organic molecules that new and more complex substances are synthesized and growth made possible.

Now we can return to the capturing of energy at surfaces. Oxygen normally reacts with the respiratory enzyme, or some other substance in the catalytic chain, embedded as an integral part of the cell structure. The energy is therefore automatically freed and caught then and there. But suppose this enzyme is poisoned by cyanide; oxidations stop, the energy flow is interrupted, the cell is unable to function and eventually dies. Methylene blue, however, can substitute for the poisoned enzyme and cytochrome, since it is able directly to oxidize

some of the molecules entering at later stages in the chain, e.g., succinic acid. But when methylene blue serves as oxidizer, it does so by virtue of carbon atoms in its molecule, which are reduced; and the total energy which is freed is quite small. The methylene white resulting from this reduction does not, of course, remain in place at the particular position where the oxidation occurred but, like all soluble molecules, diffuses about in the water of the cell; so that the great bulk of these molecules will be found at any time in the open sea rather than hugging the shore lines. But methylene white, in turn, is rapidly oxidized back to methylene blue by molecular oxygen with the attendant liberation of a large amount of energy, as with all oxidations by oxygen.

The total reaction, then, is somewhat as follows: In the unpoisoned cell, let us say 100 units of oxygen are used per hour. Adding cyanide cuts the rate down to five or less. Adding methylene blue, even in the presence of cyanide, promptly restores the rate of oxygen consumption to the original level of 100, since the poisoned step in the catalytic chain has now been substituted by methylene blue which, in oxidizing the substrate and being oxidized by oxygen, functions just as did the original system. In the final balance, therefore, the same amount of substrate has been oxidized, the same amount of oxygen has been used for this purpose and, as an inevitable corollary, the same amount of energy has been freed. But, though methylene blue can fully restore the respiration of a cyanide-poisoned cell, it does not restore its function or prevent its ultimate dissolution. The energy liberated has not been captured by the cell machine but has passed directly into heat and so been useless. The reason for this wastefulness is clear enough. The great bulk of the energy is liberated when oxygen reacts with the methylene white molecules and these were homogeneously distributed in watery solution away from the crucial surfaces and substances of the cell machine. The gasoline has burned, but not in the cylinders.
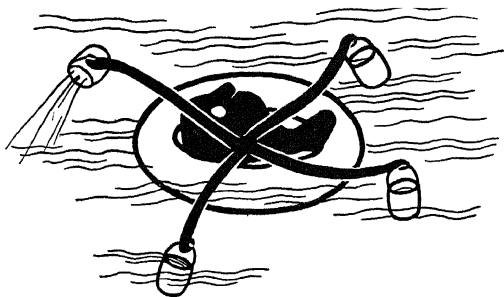
Fig. 82. Oxidations away from structural surfaces yield as much energy as do those on surfaces (compare Fig. 80), but it is squandered without doing any useful work.

Phosphate seems to be an essential cog in the metabolic machinery, particularly when useful energy is transferred from molecule to molecule or is finally released for work. The breakdown of phosphate compounds bobs up over and over again as the chemical step which is most immediately concerned with cell function and which directly yields the needed energy. Coenzyme, which is active only when a string of several phosphates is tagged on at the end of a molecule which dwarfs them in size, is vital to respiration, to fermentation, and to phosphate transfer. When hydrogen is exchanged between trioses (also combined with phosphate) the energy liberated remains in the coenzyme complex and is passed along with phosphate into a new molecule. The combination of phosphate and creatine for example, can be coupled with the formation of lactic acid—the first reaction can proceed along with, but not independently of, the second—and the energy released from the sugar is promptly stored in the creatine-phosphate. Similar coupling permits many reactions which require energy, as do most syntheses, to proceed, when other energy-yielding ones accompany them.

The further details of the belt system of the cell that passes energy on from one stage to another cannot concern us here. Unfortunately all too few of the gaps can yet be filled in. The "works" have not been seen or diagrammed, and we are almost in the position of a person trying to explain how an automobile

runs without having ever had a peep under the hood. Yet there is great hope even here, since the newer tools for study, such as X-ray analysis, which have earlier been touched upon, are rapidly uncovering these ultramicroscopic structures and molecular mechanisms. How cells handle their energy or, more properly, how they handle simultaneously their material and energetic traffic, is one of the truly fundamental problems of living things. We have already glimpsed some aspects of this in connection with the control of rate and direction of metabolic reactions and of the changes in these associated with activity, and shall return to it again in the next chapter.

The sources of the energy which cells utilize merit further consideration. The great reservoir, of course, is the mass of carbon compounds which, on reducing oxygen with the attendant electron shifts, release a steady stream of energy. Often enough, however, a sudden extra supply is needed for brief intense activity, as in vigorous muscular contraction; and oxygen cannot be supplied to the cell rapidly enough to release it by oxidations. Or, over longer periods, the oxygen supply may be inadequate or lacking. It is then still quite possible for the cell to obtain energy in less efficient ways. Two molecules of aldehyde, one oxidizing the other, yield, if not fifty-eight, at least four calories; and if this reaction occurred fifteen times as rapidly as the oxidation of aldehyde by oxygen, the same total energy would be made available. Inefficient, to be sure, but a highly useful emergency measure, like pawning
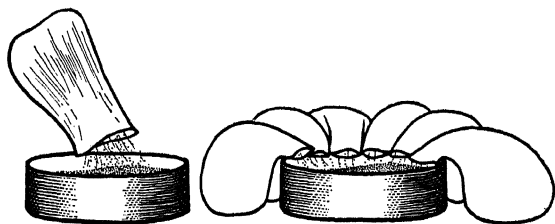
FIG. 83. One bag fully emptied or many partly emptied yield the same amount of grain; a little energy from the fermentation of each of many sugar molecules will do as well as much energy from the oxidation of one.

valuables for a small but prompt supply of fluid cash. And this, we have seen, is exactly what happens. When oxidation is interfered with, fermentation or glycolysis sets in and at a rate very much greater than the normal one of oxidation. When oxidation is restored, most of the products of glycolysis are resynthesized into sugar; the pawned jewels are redeemed with money obtained by selling one of them or something else.

The fermentation reactions are essentially the same as the interaction of the two aldehyde molecules. A molecule of glucose splits into two fractions, each with three carbon atoms and each an aldehyde (or the similar ketone). These two aldehydes then interact, dismute, through several steps instead of one, to give the acids and alcohols which are the products of fermentation. With inadequate oxygen supply, the muscle obtains the energy necessary for survival and work by this change of glucose or glycogen to lactic acid, the yeast cell similarly by changing it to alcohol; and in both cases the large quantity of chemical transfer compensates for the small energy yield to give an adequate total. In an entirely similar manner, the breakdown of creatine-phosphate or of adenyl-pyrophosphate gives a small amount of energy, and the recombination of the smaller molecules produced takes up the same amount. Throughout the course of muscle contraction and relaxation, therefore, there is a continuous balance between the energy yielded by one reaction and that required to drive up-hill to its original state some other one. This whole process has been studied in great detail by direct measurements of the heat liberated at different stages of muscle activity, under normal conditions and when one or another of the chemical reactions has been specifically interfered with.

Heat

It is no small technical problem to measure the heat produced by living cells in the course of or during recovery from a period of activity. For one thing, the actual heat freed may be extremely small; in the case of nerve hardly a millionth of a

calorie for a whole gram of it. This suffices to raise the temperature of the cell mass, even momentarily, less than one-millionth of a degree, which is far outside the range of any ordinary thermometer. Further, the heat liberation occurs often very suddenly and for only a few thousandths or hundredths of a second, and it is just these durations of change that are especially interesting. Nevertheless the problem has been solved and the actual measurements made. Instead of a thermometer, in which the expansion of mercury pushes a thread of itself up a capillary tube, the far more sensitive thermocouple is used, or rather several hundred of them arranged so as to sum their action.

This electric thermometer depends on the fact that two different metal wires, say iron and silver, attached together act like a minute battery, and the voltage of this varies with the temperature. If two such junctions are kept a degree apart in temperature, a voltage difference of some fifty microvolts, fifty-millionths of a volt, is set up between them. Conversely, if one junction is kept at some constant temperature and the other in contact with cells, a voltage appearing between them measures the temperature change in the cells. Several hundred junctions in series can develop about a thousandth of a volt for one degree. But sensitive galvanometers can easily measure one hundred-millionth of a volt or less (in such a low-resistance circuit), so that even the minute temperature changes in question can be determined accurately. Also, the response of the thermopile is rapid, unlike that of a thermometer which must be held in the mouth for a minute or longer, so that a small fraction of a second may suffice for the full potential change to manifest itself.

Muscle cells have been studied in this way far more completely than have any others, and a small straplike band of these cells, the sartorius or tailor's muscle in the frog's leg, has rendered physiology a mighty service. The sartorius is composed of single elongated muscle cells arranged in parallel order, like a package of pipe cleaners, so that the whole muscle, though over an inch long, is hardly a fourth of an inch

wide and a fiftieth of an inch thick. When carefully removed from the leg, it remains alive and apparently quite normal for days and can be made to give brief twitches or short maintained contractions over and over again. It is possible, then, to place such a muscle upon a thermopile and measure its heat production. At the same time the tension it develops and, somewhat more indirectly, the metabolic changes that occur in it as a result of activity can be determined.

FIG. 84. The sartorius muscles of a frog are placed on each side of the wires of a thermopile over the junctions of two different metals. The heat produced during contraction is measured by the galvanometer. The ends of the muscles are attached to a steel bar which twists slightly under their pull during contraction, and so rotates a tiny mirror fastened to the bar. The beam of light reflected by the mirror falls on photographic paper wound around a moving drum. The light moves as the muscle tension changes and so gives a record of the contraction force.

In an ordinary twitch, the muscle tension increases smoothly during about a thirtieth of a second and then decreases more gradually over another twentieth. At the start of contraction, there is a burst of heat given off, lasting perhaps a hundredth of a second, and again when relaxation starts another lesser burst lasting somewhat longer. The whole twitch and the heat associated with it are over in less than a tenth of a second; but the train of events started by the original stimulation is by no means over. A very feeble but prolonged and slowly fading production of heat continues for ten or fifteen minutes longer. Though the intensity of this recovery heat production may be less than one thousandth of that during the twitch, its duration is

FIG. 85. The rate of heat liberation by a muscle during and after contracting
under various conditions. (Partly after Hartree.)

so great that actually one and a half times as much heat occurs
after the twitch is all over than while it is in progress.

And what of the chemical events? No one has succeeded in
directly measuring the breakdown of creatine-phosphate or the
formation of lactic acid over periods of a hundredth of a second.
Certain indirect measurements, however, such as a change in
the transparency of a muscle or in its actual volume, are asso-
ciated with these chemical changes and give valuable indica-
tions of their occurrence. Direct chemical analysis of one
muscle before and of its mate immediately after one or several
twitches, shows the total amount of chemical change that has
occurred per twitch; and sometimes, as in measuring respira-
tion, continuous chemical data can be obtained. An increased
oxygen consumption continues over the ten or fifteen minutes
during which recovery heat is produced. The quantity of
energy that should be liberated by oxidation of the usual sub-
strates with this amount of oxygen can be calculated and agrees
very exactly with the total heat found.

The energy for muscle contraction, then, comes ultimately
from oxidation. Yet we have learned that muscle will contract
a good many times in the absence of oxygen. The heat accom-

panying a twitch is not diminished at all by lack of oxygen, though three-fourths of the following recovery heat does disappear. Clearly, during the twitch energy comes from some other chemical reactions, whereas the recovery depends on oxidations. How can the total energy be equal to that produced by oxidation, yet the first two-fifths of it come from other chemical sources? The answer we know; whatever reactions give energy at the start are reversed during recovery and the energy needed for rebuilding the original substances, exactly equal to that originally produced by the reactions, is subtracted from that freed by oxidation. The total energy balance is the same despite intermediate reactions which go forward and then back to the initial state. The last step in recovery depends on oxidations and, since the glycogen present in the muscle at the end of recovery has decreased from that originally present by just the amount that could be oxidized to carbon dioxide by the extra oxygen used, it follows that glycogen (or one of its products) is the substance burned.

Now what of the 25 per cent of the heat which is produced, in the absence of oxygen, after the twitch is over? Anaerobically, lactic acid accumulates in the muscle cells, so perhaps this long feeble heat production represents the energy released when sugar changes to lactic acid. A simple experiment confirms this, for a muscle poisoned with iodoacetic acid does not form lactic acid or manifest the prolonged anaerobic heat production. Lactic acid, then, is an intermediate product in muscle activity, formed at some stage from glycogen, along with a certain amount of energy, and later removed, with the aid of other energy obtained from oxidations when oxygen is available. All the lactic acid may be rebuilt into the sugar from which it came, some other foodstuff being oxidized; but more commonly, as we have seen, one-fifth of the lactic acid is burned to yield the energy necessary to recombine the remaining four-fifths.

It has been shown in a similar way, though with rather more difficulty and less completeness because of the very brief times involved, that the heat liberated during the twitch itself

comes largely or entirely from the breakdown of adenyl-pyro-phosphate and creatine-phosphate; and that these are then reformed at the very start of recovery, with the aid of energy obtained from lactic acid formation and ultimately from oxidations. Under favorable oxidative conditions, the lactic acid step may even be omitted and oxidation energy used directly to rebuild the phosphate compounds.

Here, then, is one case in which a fairly satisfactory picture has been reached. Most if not all of the chemical changes are known, their interrelations established with high probability, and their significance in terms of energy yield understood. Only the actual means by which this energy is transformed by the cell machinery into mechanical work—the cell devices that make the muscle fibre shorten and thicken and produce a pull—remain largely unexplained. Yet even here the solution of the problem has certainly been glimpsed, and it would be possible to explain in some detail how the stretched-out protein molecules in the colloidal micelles, arranged like minute rods along the length of the muscle cell, are caused to twist and wrinkle and so to shorten themselves and the whole structure as the result of these chemical and energetic changes which supply the drive.

Incidentally, it is worth a thought that, whereas the efficiency of a steam engine is usually about 7 per cent—that is, of all the energy liberated from the burning of coal, about one-fifteenth is successfully captured to do work, the remainder breaking down directly into heat—the efficiency of a muscle cell can be over 30 per cent—a striking tribute to the engineering skill of nature.

Energy Cycles

Now, we must face another question. If the living cells continuously obtain energy by oxidizing carbon compounds with oxygen, should not the ultimate result be a world with all the carbon as carbon dioxide, with no further energy sources, and

so no life? Or for that matter, since most carbon compounds
will slowly oxidize even in the absence of cells, how did there
happen to be such reduced substances available in the first
place? The answer lies in the other half of a majestic cycle of
change, of which we have considered only one part, and de-
pends on the action of green plants, with their precious chloro-
phyll, and of a few other insignificant bacterial cells which
possess, instead of chlorophyll, some equivalent pigment.
These colored catalysts carry on a process the reverse of
respiration. Carbon dioxide is reduced, the carbon combined
with water to give, probably, first formaldehyde and ultimately
sugars and starches, and the extra oxygen set free and restored
to the atmosphere. But if sugar on combining with oxygen to
form carbon dioxide yields energy, this reverse process must
take up the same amount. If this needed energy were obtained
from other chemical molecules, there would be still the problem
of reforming these other energy-yielding molecules. No, the
answer is of the utmost directness and depends upon the most
important invention ever achieved by living organisms.

The radiant energy of the sunlight itself is somehow en-
tangled by the chlorophyll and used to reduce the carbon to
compounds which serve as the immediate fuel of life. It is no
accident that chlorophyll is colored and that the few bacteria
which likewise obtain energy from sunlight also have colored
enzymes, for light energy is caught only when the light rays
are absorbed. If all wave lengths of the light reaching the
chlorophyll molecule were alike absorbed, it would be black;
if none were absorbed, but all reflected or transmitted, it would
be colorless or whitish; if certain wave lengths were absorbed
and others passed on, the emerging light would be white minus
red or green or blue as the case may be. But white light minus
red looks green, and leaves are green because this part of the
spectrum of white sunlight has not been absorbed. The other
rays are used, especially the red ones, and their energy is
magically hoarded in reduced carbon atoms. Red light is turned

into potato starch and the fuel that drives all living processes is so replenished.

Free Energy

This brings us to another fascinating problem that has received much thought yet remained unanswered. It requires, however, a brief excursion into physics, along paths indicated in an earlier chapter. No one has ever seen or handled energy. It is defined only by mathematical equations expressing changes in matter and is quantitatively determined in the same way. Force is measured in terms of the weight of matter and the height to which it can be lifted. Such mechanical work in turn can be directly converted into a given amount of heat, measured by its ability to raise the temperature of a known mass of water or other substance. Heat or mechanical work also have their equivalents in electrical or photic or chemical work; and the energy which makes such work possible and is expended in producing it is the same for all. The chemical energy stored in a given amount of sugar will produce so much heat,

WHITE LIGHT
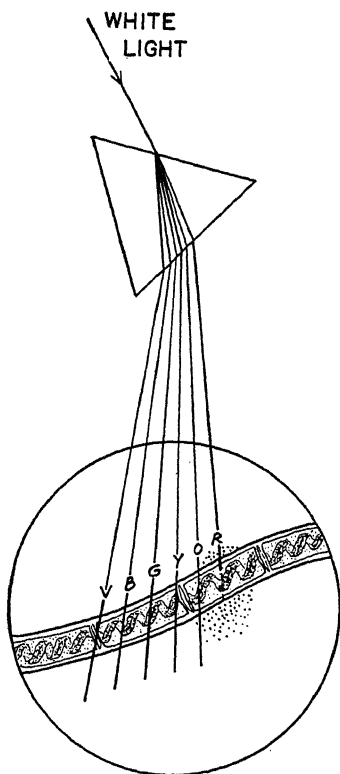
FIG. 86. Light is passed through a prism and the spectrum is allowed to shine on a filament of algæ under the microscope. The red light is absorbed and photosynthesis occurs in the cell where the energy of red light was available. As a product of photosynthesis, oxygen gas is released into the surrounding water; and its presence is shown by the clustering in that region of bacteria avid for oxygen. (Partly after Biedermann.)

or an equivalent electric current, or lift a specific weight a given distance, or produce a certain amount of light; and in all cases the energy freed turns out to be the same. No matter what intervening machinery is used to transform the energy into this or that kind of work, it appears ultimately, after the work is done, in the form of heat, and the amount of heat is constant whatever the specific path of change has been.

In fact, one of the great generalizations of science, conceived little more than a century ago and borne out by universal experience since, is that the amount of energy in the universe is constant. It may change its form, pass from one place to another, and act upon matter in various ways, but it is neither increased nor decreased. True, certain modern refinements of knowledge have shown that even this broad principle is not complete, for matter is itself an interchangeable form of energy. Neither one separately, but rather their sum, is rigorously constant through time and space. Such convulsive metamorphoses between matter and energy occur, however, under extreme conditions far beyond those which exist in living things and need not concern us here. What is important is that this Law of Conservation of Energy, or First Law of Thermodynamics, holds as rigorously for living systems as for any others. The amount of heat liberated by burning sugar directly is exactly equal to that produced when it is burned in the body. Elaborate rooms have been built in which men or animals can be kept for days and their total heat production accurately determined. The kinds and amounts of food eaten and burned in the body are also measured, and, if body weight is unchanged, their oxidation must account for the heat. The same foods burned in a calorimeter yield, to within one-half of one per cent (the limits of accuracy of the measurements), the same heat that the man produced.

But there is a Second Law of Thermodynamics, dealing with the degradation of energy. The total amount of energy does not decrease, but it may become useless. Any other form of

energy can always be converted completely into heat, but heat cannot be reconverted into other forms of energy except under special conditions. When differences in temperature between two objects exist, then the extra energy which keeps the warm one warmer can be in part used to do work and so pass through the stage of chemical, electrical, or other forms of energy. But once this has occurred, and the two bodies have reached the same temperature, no further work can be obtained from the system. Two glasses of water at 50° contain the same amount of thermal energy as when one is at 51° and the other at 49°, but in the latter case this energy can flow from the warmer to the cooler.

Or consider another condition. On a still day in August with the air temperature at 90°, the molecules composing it are dashing helter-skelter at an average speed of 19.15 miles per minute. There is an enormous energy present, but it is completely unavailable. The still warm air can do no work except as it cools and its molecules slow, so that some of their kinetic energy becomes available. But the air will not cool of itself. Of course, cold air could be brought in, but this takes work. Further, it would not be possible if the whole atmosphere were really of uniform temperature throughout the world. Suppose, however, that burning gigantic bonfires warmed the air over a large portion of the earth from 90° to 95°. The molecules are now moving at 19.24 miles per minute, or with an increased average speed of five miles per hour. They contain more energy, but it is still as completely unavailable as before. But if, instead of warming the air, it were set into motion, so that a wind blowing at five miles an hour came up, such air could turn windmills, sail boats, and in general perform great feats of mechanical work. Yet the energy is identical in the air at 95°, whose molecules are moving at an average velocity of 1,154 miles an hour, and in the wind at 90°, whose molecules move 1,149 miles an hour plus the wind velocity of five miles an hour. Just where is the difference?

RANDOM   DIRECTED
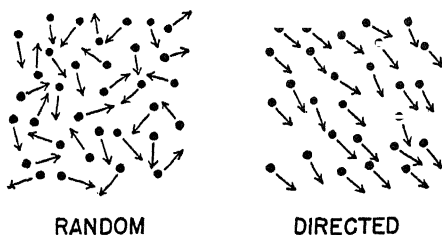
FIG. 87. Molecules moving at random, or with a general direction as in a strong wind. The total amount of movement is unchanged but the wind can do work.

In the first case, the molecular movements are completely random, the system homogeneous, and the energy unavailable. In the other, though most of the molecular movements are still perfectly random, some, indicated by the wind, are not. The average molecular movement of five miles an hour is all in the same direction. To this extent the system is not homogeneous or disorganized but has a true organization. It is reminiscent of a trick used in steer wrangling. A refractory animal is tied to a well-domesticated burro and the two then released. The strong steer dashes off, banging the small donkey along with it, and disappears. But after some days the two arrive safely at the corral. The great force of the steer is expended in a random fashion, the feeble one of the burro is directed—every step it is able to make is turned toward home.

Left to itself, an organized system inevitably tends to become chaotic. The wind dies down, the molecules jostle at random with the same speed as before, but the air now is merely a little warmer. The essence of the second law of energy is that the mixed-upness of things increases. Systems do not spontaneously achieve organization but chaos. As an organized system becomes mixed up, its energy, instead of being free and able to do work, becomes useless heat energy. Within the entire scope of man's experience the change goes forward only in this direction. Energy is becoming ever less free and organized systems ever more disorganized.

True, organization is continuously being produced, especially by man, yet always at the expenditure of free energy from other organized systems. If this is true everywhere, then the universe is running down like a wound-up clock and will some day be completely chaotic and homogeneous, with no energy available to work or bring about change. This somber picture of a dead universe may perhaps be premature, for man's experience is limited to a minute portion of the time, space, and states of existence; perhaps in other places or under other conditions, free energy is re-formed. In principle, this is simple enough.

If, somehow, the random molecular movements of heat could again become organized, the amount of energy would not be altered but that present could again do work. Suppose an infinitesimal demon, as the great mathematical physicist, Maxwell, argued, were to close a shutter each time a molecule approached from the left, so that it rebounded from the surface, and were to open the shutter every time a molecule approached from the right, so that it could pass through. Then, without any work being done, for of course the shutter is frictionless, the free energy would be increased, an ever
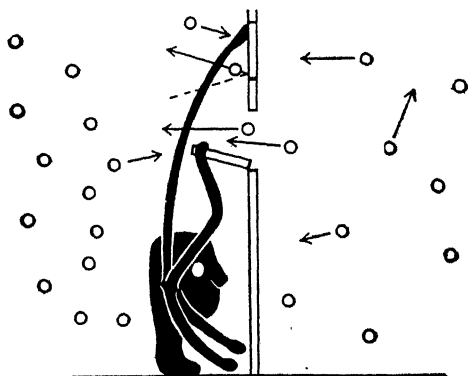
FIG. 88. A "demon" could overcome the second law of thermodynamics and turn mixed-upness into order by opening frictionless ultramicroscopic trapdoors in a membrane so as to let molecules coming from one direction pass through, but closing the doors to block molecules moving in the opposite direction.

greater number of molecules would move to the left, in fact, the wind would be restored. To choose another example: Place a membrane with openings of molecular size between equal volumes of two different kinds of gas. The molecules will gradually pass and repass through the partition from either side until an equal mixture of the gases is present on both sides. While reaching this diffusion equilibrium, work can be done. In the mixed and random condition the molecules still wander about; is it not conceivable that chance could bring them again to the original organized state?

Suppose, for example, that there were only two molecules of A to the left of the membrane and two of B to the right. With each free to move in the total space, the chance for each A molecule of finding itself in the left chamber or for each B molecule of being in the right one is, at any instant, one-half. By chance, then, there is one possibility in sixteen $(1/2 \times 1/2 \times 1/2 \times 1/2 = [1/2]^4)$ that at some time the molecules will reachieve their original distribution—not such outrageous odds, and plenty of time to play them. But when we substitute for a hypothetical case of two molecules a more real one, say a million times a million on each side (about the number present in a cubic millimeter of the most perfect vacuum man has made), then the chances of the molecules' sorting themselves out after once mixing are $(1/2)^{10^{12}}$. Doomsday will happen first! Still, the demon, if he but existed, could open and close the holes as each kind of molecule approached and in no time at all have all in the proper pen.

But what has this to do with the living cell? Is it possible that within it reside just such demons? There are present, to be sure, the very kind of membranes in question, ones which let certain molecules and ions pass and not others. It is even true that certain plant cells placed in sea water proceed to pass potassium ions from it into their central sap even though this already contains the ion in a hundred times greater concentration. Cells are the most superlatively organized objects in

the universe and they show growth, the astounding ability of taking other unorganized materials and building them up into like organized systems. Can living things, then, transcend the second law? Is this a basis for "vital" action?

Some have thought so, and possibly the time has not yet come to consider the question closed. None the less, the probabilities are strongly against it. When living cells sort out ions, build structure, and increase complexity, they do so only with the aid of free energy obtained from other sources, not as Maxwell's demon would by clever frictionless acrobatics. For each increase in free energy built into the organization of living things a much larger amount of the free energy of their foods is used up. If oxidations are stopped in the plant cells that are concentrating potassium, the accumulated ions promptly leak out again; growth, even more than maintenance, is impossible when energy-yielding reactions are suspended.

For this reason, if no other, meat and dairy products must always be more expensive than agricultural ones—the free energy locked in the substances formed in animals has been captured only by the expenditure in their bodies of much more free energy, originally stored by the plants. The whole web of life is, in its simplest terms, a struggle for free energy, whether it be between shrub and tree for a place in the sun, between locust and rabbit for the energy-yielding compounds of leaves, or between lion and tiger for the flesh of an antelope. Free energy all living things must have, for without it change is petrified; and life is change.
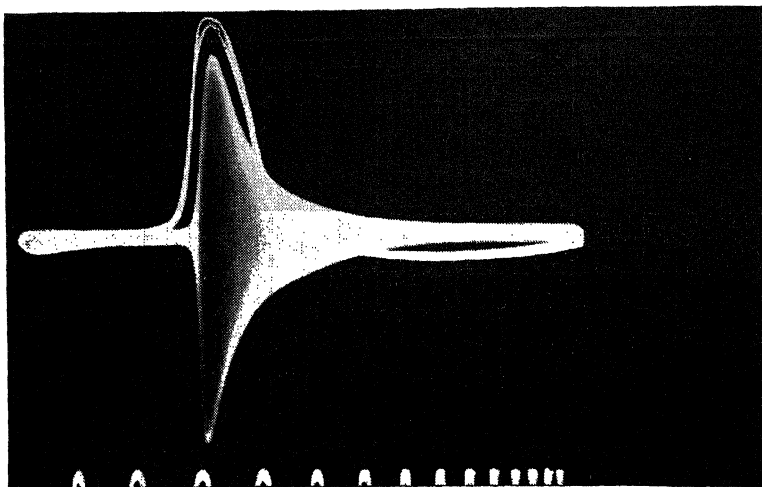
Chapter Eight: Action and Its Consequences—
Irritability and Behavior

The old parable has it: the loss of a nail caused a horse's shoe to drop off, lamed the horse, and prevented his rider, the general, from reaching the scene of battle. The battle was lost, the war was lost, and the course of history was deflected "all for the loss of a tuppenny nail." So we are taught that great consequences grow from tiny causes like great oaks from tiny acorns. Of course it is not so simple as that; only when the myriad other conditions upon which the end result also depends are fulfilled in some particular way, can such a sequence be traced through to the particular factor that interests us. If the other nails in the shoe had held, if another horse had been available, if a disaster had afflicted the opposing army, if the general had been near his troops, if this, that, or the other condition had been different, the "tuppenny" nail would not have achieved a literary immortality. Still, when everything else is properly set, the small beginning can produce an impressive result. When the gun is properly loaded, the small

trigger pull does initiate a much greater activity and energy liberation. If the bullet be properly aimed into a dynamite dump, again the small energy it introduces initiates an incomparably greater change.

Such magnification of energy change is often called trigger action. It is characteristic of organized systems, most especially of living ones, and is the theme of this chapter. Cells and organisms do not remain primarily quiescent and inactive, sluggishly digesting their nourishment to the sole end of continuing a nourishment-digesting existence; they are active in the world about them, even the most placid and uninspiring of slimes and molds. Besides the resting metabolism with its physical and structural consequences, there are the additional capacities of the cell to do something when something is done to it. It can respond to a stimulus; it can vary its behavior consequent to a change in its environment; it can exhibit large changes in the amount and direction of its energy flow, following the application to it of minute amounts of energy from the outside. It is, in short, the loaded gun aimed at dynamite, and a trigger touch can cause a great explosion.

But living things do not just blindly explode their energy stores in response to environmental vicissitudes; they also direct them. The organism must maintain its complex integrity against the hostile forces of confusion, it must fend the "slings and arrows of outrageous fortune" and so counter or adjust to the surrounding environmental whirl that it remains itself and yields as little as may be necessary. The more it changes the more it remains the same. True, over longer times, covering the evolution of the race or even the development of the individual, progressive change is a vital part of the life picture, and again along regular rather than random lines. But from moment to moment the individual acts to preserve itself in its current state.

To environmental changes, or stimuli, living things respond
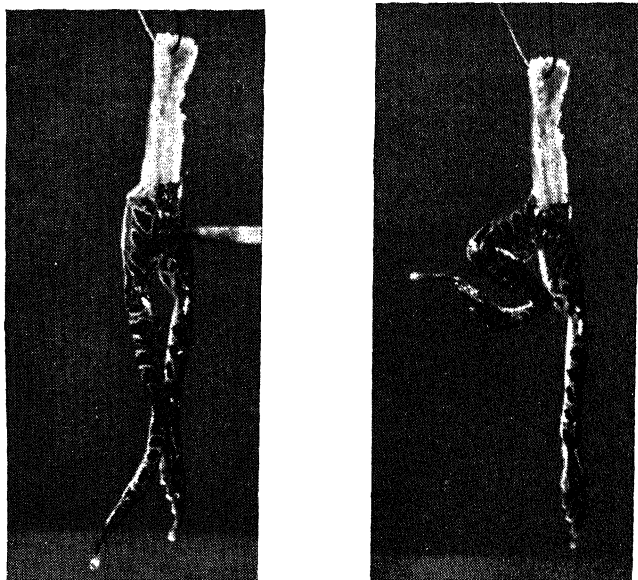
FIG. 89. A living system responds adaptively to a stimulus by attempting to remove the disturbance. The completely isolated legs and spinal cord of a frog still give a seemingly intelligent, but completely reflex and automatic, response to the presence of irritating vinegar on the skin. The leg makes a vigorous and continued effort to scratch away the irritant. (From the film, *The Nervous System*, by Gerard.)

adaptively, they make such changes in their behavior as most effectively preserve the status quo. This is true for the increase in fermentation with which yeast cells respond to interference with their oxygen supply and for the frantic rush of the deer on scenting the leopard. In each case the temporary change in activity helps preserve the possibility of continued existence of the organism. Comparably, the lobster caught by his claw will forcibly shed this appendage and so preserve the rest of him. So much, then, is obvious: living units are sensitive to stimuli and respond, often to the most minute environmental change, with striking and directed activity. They manifest adaptive amplification. But what is the mechanism determining such

behavior, and how is the placid metabolic stream diverted from its resting course?

Stimulation

One important clue comes from a study of the stimulus itself. The most elaborate array of environmental changes can stimulate: a mechanical touch, a temperature change, a flash of light, the regular mechanical vibrations of sound, a change in osmotic pressure, a pulse of electricity, altered chemical composition, or what you will. Incidentally, either an increase or a decrease in amount may be equally effective, but this part of the story belongs farther on. What is important now is that, whatever the qualitative character of the stimulus, certain quantitative relations apply to it. Even more, these same relations are true no matter what cell or organism is being stimu-
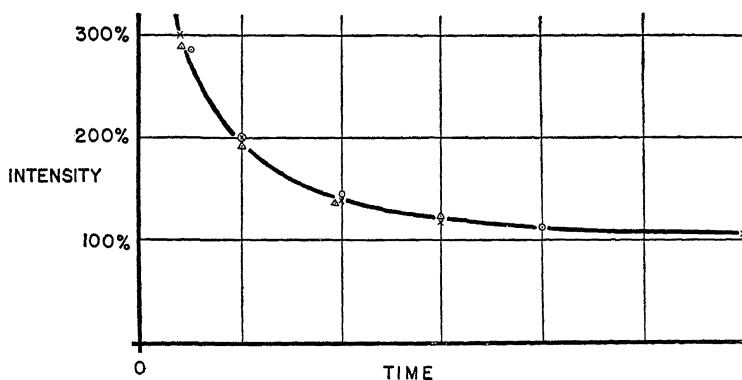
FIG. 90. The intensity of a stimulus of long duration which will just excite is called 100 per cent, whatever its absolute value for different tissues. Then, as the duration of the stimulus is made shorter its intensity must be increased if it is still to be effective. The times involved vary enormously for various tissues, but when the scale is adjusted at one point all the others come together also and a single curve results. The experimental results are shown for three kinds of muscle: frog's leg muscle (x); frog's stomach muscle (△); and snail's retractor muscle (o). The time scales, given by the whole width of the plot, are: leg muscle, 0.003 second; retractor, 0.13 second; stomach, 6.0 seconds. (Partly after Lapicque.)

lated. The numerical values are different for each case, but the following "laws" are true whether the human eye is stimulated by light, a frog muscle by an electric current, a paramecium by a mechanical touch, or an egg cell by acid.

Some minimal amount or intensity of stimulus is required—feebler currents, dimmer lights, more dilute acid will have no effect. Only when some threshold intensity is reached or exceeded does a response follow. In many cases, indeed, once a threshold strength has been reached, more intense stimulation has no additional action, a threshold stimulus and a far stronger one eliciting identical responses. Further, the stimulus must last some minimum length of time. If too brief, just as if too feeble, it will not excite. Finally, there is a regular relation between the strength and the duration of a stimulus required to make it of threshold value. The greater the strength, the shorter the necessary duration, and vice versa; so that over quite a wide range the product of strength and duration is a constant. Only at the extremes does this no longer hold, for stimuli below a certain strength will not excite however long they last, nor will those shorter than a minimal duration however strong they be.

The product of strength and duration is, of course, the total quantity of stimulus applied, and all this can be roughly summarized by the statement that a constant amount of energy must be applied to stimulate effectively. The actual amount of energy varies, of course, for each stimulus and each cell. The retina can respond to less than a millionth of a meter-candle of light (the brightness of a white card illuminated by a single candle a fifth of a mile away) lasting a hundredth of a second; whereas the clam may take a light of five meter-candles lasting a second to make it pull in its siphon, and the sea squirt will not respond to a thousand meter-candles unless it continues for five seconds. A current of less than a tenth of a milliampere lasting for a ten-thousandth of a second can stimulate a nerve fibre in a frog's leg, while one of one milli-

ampere lasting over a second is required to cause contraction of a muscle cell in its stomach.

In each case, however, whatever energy is required to stimulate at one intensity and duration will also do so at others. (Actually certain optimal times require minimal energy because of complicating factors.) Electrical stimuli have been used far more extensively than any other type for experimentation be-
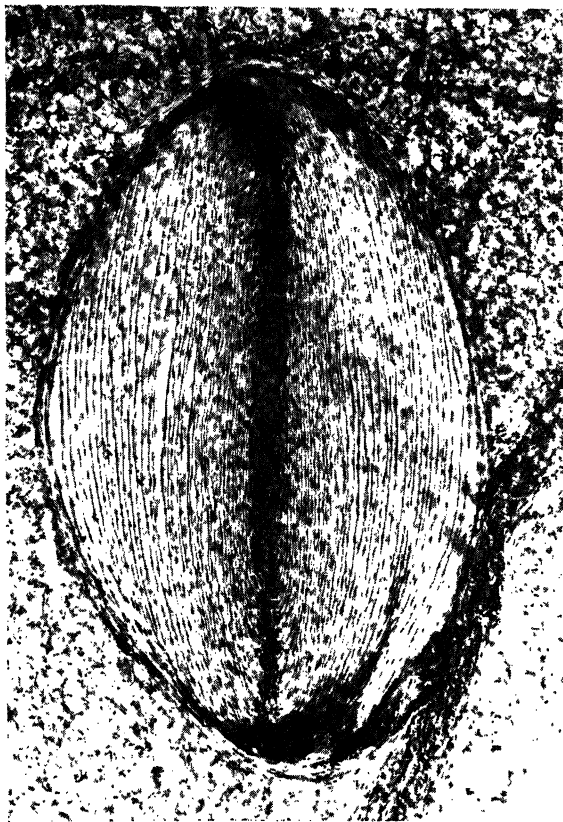
FIG. 91. Microphotograph of a single touch receptor enlarged 100 times. The nerve fibre from it is seen on the right side running out at the bottom of the picture.
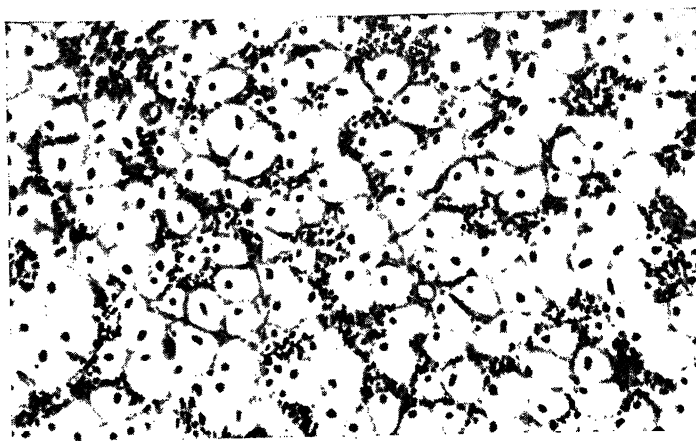
FIG. 92. Microphotograph of a cross section of a nerve showing medullated axones (black dots are axis cylinders and the white zone around a dot is the layer of myelin), and non-medullated ones (clusters of fine black dots). (After Ranson. From Maximow and Bloom's *Textbook of Histology*. Copyright W. B. Saunders Company.)

cause they can be so easily and accurately controlled and measured; and nerve fibres, supremely specialized to transmit excitation, have been most intensively studied with their aid. It will be simpler to continue the discussion in terms of these, understanding that other agents act in identical or analogous fashion on other structures.
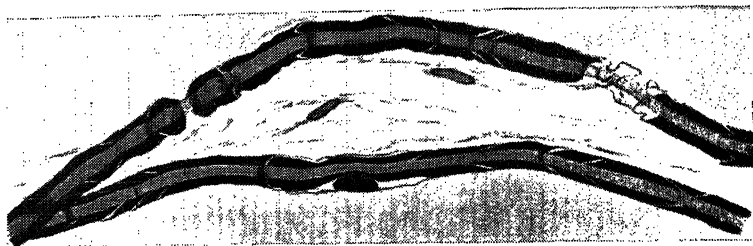
FIG. 93. Two medullated nerve fibres teased out from a nerve. The black myelin sheath shows slits and nodes, the wider interruptions, which are normally present. (From Maximow and Bloom's *Textbook of Histology*. Copyright W. B. Saunders Company.)

The nerve fibre is a hugely elongated process of a nerve cell, rather like the single thread of silk from the end of which a spider hangs. In cross section, it is orthodox enough—most have a diameter under one-hundredth of a millimeter, but its length is outrageous for a single cell—over a meter in many instances. Like other cells, it is covered by a membrane enclosing more or less homogeneous protoplasm; and we shall not be seriously in error if we think of the surface film as a thin regular hollow cylinder. The membrane is semi-permeable, some ions and molecules being unable to pass, for the reasons we learned earlier. Direct chemical analysis of a bundle of nerve fibres shows, for example, a much higher concentration of potassium and a lower one of sodium than is present in the tissue fluid which surrounds them; direct evidence of the membrane's impermeability to these ions.

This property makes possible another important condition of the surface; it is polarized. An excess of positive ions is accumulated around the outside and of negative ions within, so that the whole is like a tiny charged battery (or better, condenser). When the membrane is broken at some point the battery is discharged and the ions flow together to become a neutral mixture. Then the outside of a normal fibre, with its halo of positive ions, when connected through a galvanometer to the injured region, should act as the plus pole of a battery and send a current through this measuring instrument—and it does. It has even been possible, in larger muscle or plant cells, to place fine wires on and in a single cell and so more directly measure the voltage across the membrane. It is considerable, over a twentieth of a volt in some cases. We are hardly surprised to find, further, that this potential is dependent on energy supplied by the metabolism of the nerve fibre and falls away in a few hours when the nerve is deprived of oxygen.

When one end of a nerve is stimulated, a message or impulse travels rapidly along it. Normally the stimulus originates in a sense organ, or another nerve cell, and ends at another neurone

or an effector, such as muscle. But for convenience in study, a point along the nerve can be stimulated electrically and the muscle twitch used to indicate the arrival of the impulse at the end. By measuring the time between stimulus and twitch when the stimulus is applied far from the muscle, and again when it is moved closer, say by three centimeters, the speed of travel along the nerve is obtained. The time difference is only one-
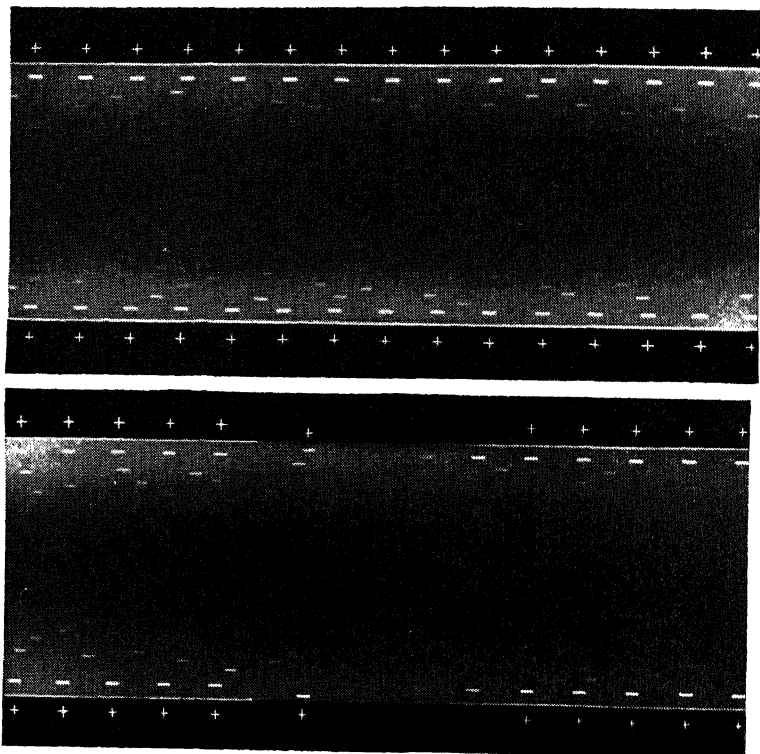
FIG. 94. The membrane of the resting nerve fibre (above) is polarized, with excess positive charges outside and excess negative ones within. When an impulse travels along (below), the membrane loses its electrical resistance and allows the charged ions to flow together and so discharge the polarization. The fibre promptly recovers, however, and restores the resistant membrane and repolarizes it. (Diagram from the film, *The Nervous System*, by Gerard.)
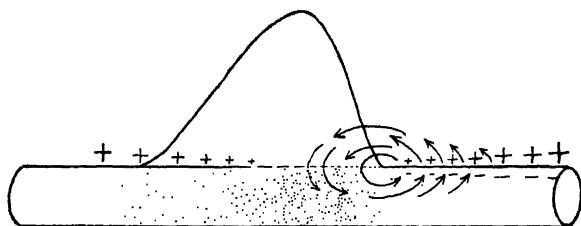
FIG. 95. Diagram of impulse in a nerve fibre, showing action potential, membrane depolarization and repair, and local currents. (Modified from Lillie.)

thousandth of a second, for the nerve message zips along at thirty meters a second, a mile a minute, but can be measured quite accurately. (This is the speed in the main leg nerve of the frog. In man it is four times faster, mainly because of his higher body temperature. In more primitive nerves, or when transmission is along unspecialized protoplasm, conduction may be far slower—a meter a second in the jellyfish and hardly one-hundredth as fast in a sponge.)

One of the early triumphs of physiology was the demonstration, almost a century ago, that an electrical charge accompanies the nerve impulse. As the active front reaches any region of the fibre this portion becomes less positive than the inactive stretch ahead or the recovering one behind; and a galvanometer, or better, a rapid oscillograph, will record a negative dip as the impulse passes the point on the nerve to which it is connected. Is this negative potential wave, like the negativity of an injured region, a sign of the local breakdown of the polarized membrane? Let us return to the electrical stimulus.

A constant quantity of electricity means a given number of ions flowing, so an electrical stimulus is effective when some fixed number of ions has been caused to flow through the protoplasm. But the membranes do not permit one or another class of ions to pass through. How, then, can the applied electrical stimulus cause ions to move across them, and why should
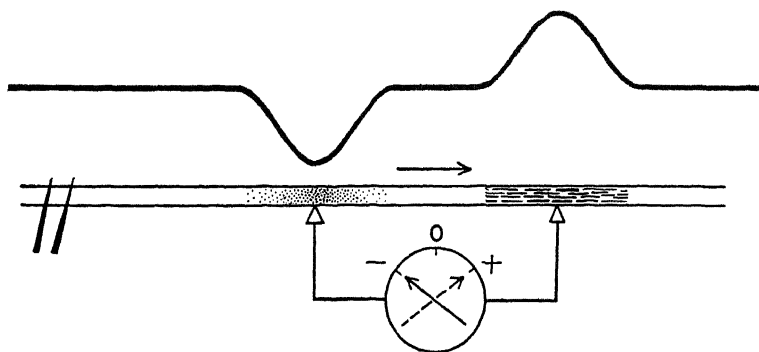
FIG. 96. Diagram of nerve fibre, stimulated at the left end and connected at two points to an oscillograph. When the traveling nerve impulse was over the first connection, or lead, this became negative compared to the far one (dotted impulse and pointer reading; downward dip in the potential curve above). Now the impulse is over the second lead and all the potentials are in the reverse direction.

FIG. 97. Oscillograph tracing of the action potential of a frog's nerve. A rise in the curve indicates a more negative potential. Time, in 1/1000th second, is shown below. The record on the left is obtained when the nerve impulse reaches one lead to the oscillograph but is blocked before the second lead. The record on the right shows the impulse making first one lead negative and then the second one negative, which is equivalent to the first one becoming positive.

such movement lead to excitation? Could an increased polarization cause a physiological spark to fly, as an electric spark leaps between two insulated conductors with sufficient potential difference between them? This is easily tested.

With a long cell, like the nerve fibre, the positive and negative poles of a stimulating current can be applied a considerable distance apart. Positive ions flow from the positive pole toward the negative, negative ones in the opposite direction; both moving through the salt-containing fluids in which the cell is bathed. At the cell membrane, near the positive pole, if ions cannot freely penetrate they will tend to pile up, positive ones against its outside and negative ones pulled along within the cell interior, against its inside. Since the membrane is already polarized with an excess of positive ions outside and of negative ones within, the polarization will be increased in the vicinity of the positive pole. By the same reasoning, it must be decreased near the negative one. Yet the response does not start under the anode (positive pole); on the contrary, it is at the region of the negative pole, where depolarization occurs, that excitation is effective. The time which elapses between the stimulus to the nerve and the response of the attached muscle is shorter when the negative pole of the stimulating current is close to the muscle than when the positive pole is.

Conduction

The rest of the story, or at least that part of it having to do with the spread of excitation through the cell, is relatively simple. The accumulation of negative ions outside the membrane and of positive inside or, more accurately, the decrease in the initial excess of positive without and of negative within, produces the local excitation. Some change in the membrane results—perhaps a change toward the sol state. It obviously does become more permeable, more or less completely broken

down, and no longer offers a barrier to the passage across it of the various ions. Those previously accumulated on opposite sides of the membrane are now no longer insulated from one another in the vicinity of the leaky region but can discharge through it, again as a battery discharges when its poles have become short-circuited.

The photograph (courtesy of K. S. Cole) at the opening of this chapter shows the experimental proof that the membrane becomes more permeable when stimulated. The thin white line in it is the potential sweeping along with the nerve impulse; and the white band measures the electrical resistance (impedance) of the membrane, becoming wider as resistance decreases. The resistance falls promptly after the action potential arrives at the portion of the nerve under study—how promptly you can judge from the loops at the bottom of the picture, which appear at intervals of 1/1000th of a second.

As each new region of the cell membrane is discharged and depolarized it, in turn, undergoes the same changes as did the portion originally depolarized by the electrical stimulus. Still more distant areas can now discharge through these newly permeable ones, the changes are again repeated, and the depolarization spreads progressively over the entire membrane surface. This propagated electrochemical membrane change, which sweeps from a stimulated point to the far end of the nerve fibre, is the essence of the nerve impulse.

This picture is clear and well composed, except for two large dark lacunae in the canvas: just how is the membrane change brought about by depolarization; and how does the spreading excitation set off the other events in the cell which result in effective behavior—contraction, secretion, division, or whatever the appropriate action may be? Even within the nerve fibre, whose sole duty is to propagate the excitation from one region to another of a complex multicellular organism, other changes follow excitation. Increased metabolism is shown by a faster rate of respiration and of heat production; and phosphate compounds break down.

Further, for nerve as for other cells, recovery must follow transmission, the membrane must be restored and repolarized, and the physico-chemical machinery be wound up in anticipation of another response. In nerve, recovery is sufficiently advanced in a thousandth of a second so that a feeble response can be obtained this soon after a preceding one, and it is complete, in the sense that another full-sized response can occur, within a hundredth of a second; yet chemical and physical reverberations continue to echo for half an hour or more. These changes in the nerve, which only conducts, are quantitatively very small in comparison with those of muscle and like cells which, in addition to spreading the alarm, do something about it. How, then, does depolarization produce a membrane change and how does this set off the other cell processes?

At both points we must guess. The changed distribution of ions about those molecules and micelles which constitute a membrane might alter their charges and electric fields and so increase their mutual repulsion and make them push apart. This would open wider water channels through which other ions could more freely pass. So Arnold von Winkelried broke the ranks of the Austrian lancers by hurling his body onto their serried array of spearheads to make an opening through which his comrades could rush. Or the changed ion concentration might lead to actual chemical changes in the membrane molecules and so even more drastically rupture the impermeable barrier.

Either or both types of change could readily occur; and plenty of cases are known in which the orientation of molecules is altered or chemical reactions between them hastened or stopped by a change in the concentration of particular ions. A local increase of potassium ions, for example, tends to disperse colloids, which increases membrane permeability and accelerates certain oxidations. Calcium ion combines with phosphate to form an insoluble compound and, by removing it, might slow the many metabolic reactions in which it is involved. Further, there is a definite time interval, from

0.00003 second in nerve to over 0.01 second in slow muscle cells, between the application of the stimulus and the beginning of the response; an interval perhaps necessary for chemical changes to progress in the membrane.

The problem at the other end of conduction is perhaps quite similar. When the excitation has spread through the cell and membrane barriers have been partly disrupted, substances previously kept apart may mix, inactive enzymes be liberated, changed ion concentrations accelerate this or the other reaction, and the chemical merry-go-round sent spinning. In one situation, at least, there is very convincing evidence of just such a chemical link in the sequence of changes from membrane breakdown to response.

Where nerve cells act upon muscle or gland cells special chemical substances help to carry over the activity. The nerve impulse sweeps to the end of the nerve fibre and there liberates a minute amount of a chemical which, in turn, causes the attached muscle to contract or the gland to secrete. Each type of nerve liberates a specific substance; two of these have been shown to be freed when nerve impulses reach the junc-
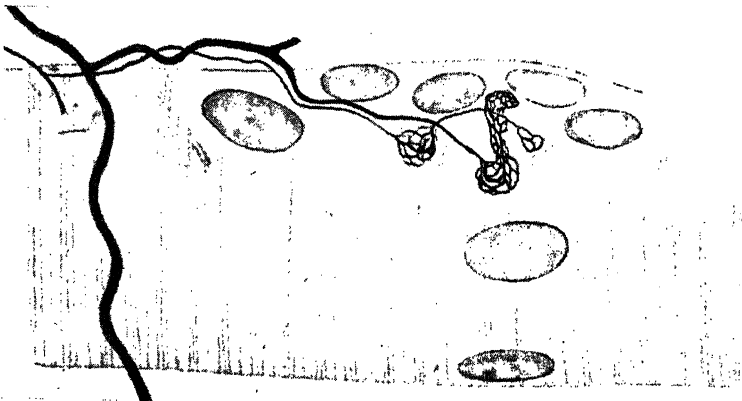
FIG. 98. Ending of a motor nerve fibre on a striated muscle fibre—the neuro-myal junction. (From Maximow and Bloom's *Textbook of Histology*. Copyright W. B. Saunders Company.)

tion of nerve with muscle or other cells, have been isolated and identified, and turn out to be simple and well-known compounds. These neurohumoral substances, as they are called, are of course formed or liberated on the spot as the excitation process reaches the nerve endings. They lead, in turn, to contraction of the muscle or equivalent activity of other effector cells, even when introduced independently of any nerve stimulus. Here, then, is a clear-cut chemical link between the membrane change of excitation and the final active response of the cell. In this case, however, the link is not merely between two events but also between two different cellular units. Possibly for this very reason the substances appear outside of the cells rather than within them—a circumstance which certainly favored our discovering them.

Contraction

The final response of the cell is the execution of its physiological function. This is fairly unique and highly characteristic for each type of cell, and each instance represents a separate problem in cell physiology. Certain characteristics are commonly present, such as increased metabolism, but even here the generalization is not perfect, for some marine egg cells actually decrease their respiration when fertilized, which is, of course, a type of stimulation. Those properties common to the conduction process are, however, indeed fundamentally alike in all cases where this occurs.

Thus, as for the nerve impulse, the contraction of a muscle induced by an outside stimulus, which starts an excitation spreading through the muscle membrane, obeys the all-or-none law. The response, that is, does not vary with the stimulus strength but, like the flare of a struck match, either is produced in full magnitude or fails entirely. Further, after a conducted impulse has passed, a small but perfectly definite time is required before recovery is sufficient for a second one to suc-

ceed it. The actual contractile mechanism in a muscle cell, on the contrary, does not conform to either of these attributes. The degree of shortening varies progressively with the strength of stimulus applied directly to the contractile mechanism; and, by means of a second stimulus, an additional shortening can be superimposed upon one still in progress.

Just how a stimulated muscle contracts has been hinted at several times. It does not significantly decrease its volume but becomes shorter and thicker. Clearly some internal organization makes it act differently in its various dimensions. The whole mass is more or less elongated to begin with, of course, but it doesn't follow that the response in this axis should be unique. A long lump of dough does not shorten when baked or handled. The muscle, to be sure, is built of thousands of parallel fibres commonly stretched out in this long dimension; but these are still only more elongated cylinders and face the same problem as does the whole muscle. A finer organization is called for.

This is partly revealed under the microscope, which shows alternate light and dark bands or discs piled on each other to make the fibre length, and changing their absolute and relative thickness during contraction. More important than their appearance in ordinary light is their behavior in polarized light, for the dark discs twist this light much as do certain regular crystals. The details of this story are beyond us here, but it is known that this property depends largely on the presence in the dark discs of many ultramicroscopic rods, colloidal micelles, oriented with their long axes all parallel to that of the fibre. Further, X-ray studies show each micelle to be built of parallel chains of extended protein molecules.

We have seen that in contraction these protein molecules become more coiled up, like a stretched spring that is released, and this of course puts tension on the ends of the micelle and makes it shorten. The entire company of rods, all collapsing together, sum their tensions at the ends of the muscle fibre, and

the many fibres pull the muscle ends toward one another. Or try to; for if these ends are held rigid then the individual micelles simply shorten somewhat, dragging their ends together through the viscous surrounding protoplasm.

The coiling of the proteins is due, in turn, to chemical changes, perhaps nothing more elaborate than an altered concentration of hydrogen ions, which result directly from the excitation that has swept along the fibre membrane. If the acidity is changed so that the protein is closer to its neutral point, not acting as either acid or alkali, then its own basic amino groups and acid carboxyl groups combine with each other. They must come closer together to do this, and so the molecule coils up more tightly. The real work must then be done to get the molecules stretched again—just as the laundress puts curtains or socks on stretchers to "unshrink" them; for fabrics are also made of fibres with elongated molecule chains which coil up when wetted and dried. In the muscle this work comes from the many chemical reactions accompanying recovery, ultimately from food oxidation. But the immediate relaxation depends on energy from the breakdown of phosphate compounds and the restoration of the normal acidity and other conditions within the protoplasm.

Rhythms

One important question has been neglected in all this discussion of rest and activity of cells. Is there always a sharp line of distinction, one of those rare perfect boundaries, between the two states, or may there be a more or less imperceptible or fuzzy gradation from one to the other? The answer is clear enough for a nerve fibre conducting a single impulse in response to a stimulus, or in a muscle executing a single twitch: the line is sharp. But what of the situation when a muscle, following a number of contractions, remains considerably shortened, though no further stimuli are

applied? Since this state of maintained shortening or con-
tracture will normally disappear after some time, it can be
called fatigue, and the changes which the muscle manifests
during the interval, those of recovery. Is the fatigued and re-
covering muscle, then, in a state of rest or of activity? And if
the answer here is not sufficiently uncertain, what of that semi-
permanent change which follows repeated activity and which
we call training? Still more, how shall we interpret the chang-
ing state of a cell, especially one varying regularly and re-
currently so as to yield a rhythm, when this occurs without any
equivalent change in the environment; when the cell surround-
ings, in fact, are held constant?

Is the rhythmically beating heart active or at rest, or is it at
rest with interposed periods of activity? This latter is reason-
able enough for the heart, whose muscle is relaxed for nine-
tenths of a second and contracted for one-tenth. But a muscle
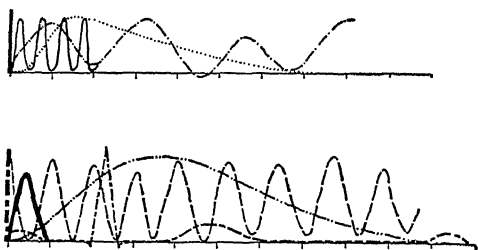
FIG. 99. Various tissues respond, once or rhythmically, at very different speeds,
even at the same temperature; and any one tissue speeds up as it is warmed.
In the upper graph of response against time, each interval on the horizontal
time axis is 2 seconds; in the lower graph each interval is 0.1 second. The
responses shown are: .... the single contraction of smooth muscle
in the cat's urinary bladder; _.._.._ the rhythmic contraction of smooth muscle in
the rabbit's intestine; ⸺ the beat of a frog's heart; ⸺ the twitch of a frog's
skeletal muscle, too quick to follow here. With time spread out 20 times, below,
the muscle twitch ⸺ is clear; the heart contraction _.._.._.._.. seems very
"slow"; but the electrical waves accompanying the heart contraction (electro-
cardiogram) — — — – — are quick enough. The electrical waves from the
brain — — —, a regular rhythm at 10 a second, can be followed well enough;
but the action potential of a nerve impulse ._._._. lasting one-thousandth of
a second, is still too fast for its form to be shown.

cell in the intestine slowly and smoothly contracts and relaxes and contracts again, to produce a continuous up-and-down wave of shortening and lengthening which may require three seconds or more to complete one cycle, and then continues on to the next so smoothly that one could equally well call the change from short to long to short, or from long to short to long, the single response. Is such a cell at rest or active? In exactly the same way, the bodies of nerve cells show a smooth continuous rhythm of potential change when they are at "rest" in a constant environment.

Yet all these changes in cell state, whether very slow and progressive or rapid and rhythmically repeated, are very susceptible indeed to environmental changes. The heart beats regularly and continuously in a salt solution containing sodium, potassium, and calcium, but remains relaxed when the last of these is omitted. It is fair to conclude that one environmental factor necessary to the beat of the heart is the calcium ion. Is this substance, then, a "stimulus" to the heartbeat? The rate of the electrical rhythm of brain cells increases with rising temperature, slows and stops as the temperature is lowered. Is temperature a "stimulus" to the electrical beat?

All cell states, whether held at some constant level or rhythmically changing, are dependent upon physical, chemical, and biological factors in the environment which constantly play upon them. Temperature, electric currents, light, osmotic pressure, acidity, salt balance, the presence and quantity of dozens of other agents, all act upon and control the state of the cell. Are they, then, stimuli? And if considered to be such, what about our picture of a stimulus as a *change* in the environment? These conditions may remain constant over long times and yet continue to exert their influence. Again the problem falls into the limbo of relatives rather than absolutes, of continual, rather than quantal, changes.

When a constant current strong enough to excite is allowed to flow through a cell, say a nerve fibre, one response is set
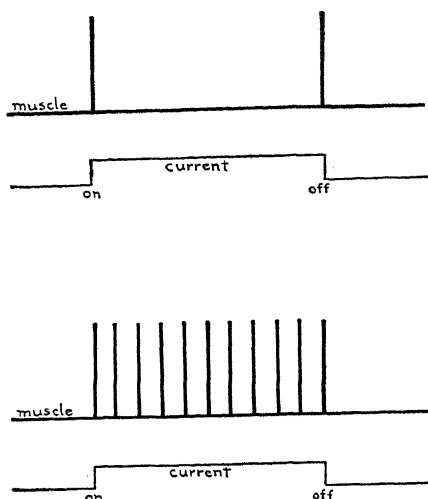
FIG. 100. Above, muscle in balanced salt solution. Twitches occur only at the start and stop of an electric current. Below, same muscle in a solution containing less calcium than normal. Rhythmic twitches continue while the current flows steadily.

up as the current is initiated, another when it is discontinued. A constant current stimulates at make and at break but not while steadily flowing. This is one extreme. At the other extreme, perhaps, are the so-called tropistic responses. The growing root of a plant, for example, continues to bend toward the pull of gravity steadily and continuously throughout its growth, or until it has become directed straight toward the center of the earth. And all intermediate degrees of response to constantly maintained conditions can be found. If the current passed through the nerve be made rather stronger, instead of one impulse two or several may be discharged at its start and a number more at its close. If, in addition, the calcium ions surrounding the nerve be somewhat decreased, responses may continue intermittently or even quite regularly throughout the passage of the current. Finally, with still less calcium, similar rhythmic discharges may continue with no current at all.

Consider another example. When light falls on the receptor cells of the retina they become active and initiate impulses in the attached optic nerve fibres. Here, as elsewhere, there is a burst of activity when light starts and again when it stops. Subjectively this is well borne out, since an increase or decrease in illumination produces a much stronger sensation than does a steady light. Nevertheless, we do continue to see during constant illumination and impulses do continue to pass along the optic nerve. Actually several different and specific kinds of receptor cells have been discovered in the retina.

One type sets up a single or very few impulses in its attached nerve only when light is turned on and shows no further change while the light is continued or when it is turned off. Another responds similarly briefly only at the moment illumination is stopped. A third enters into activity both at the beginning and at the end of, but not during, the light exposure. Still another receptor shows no especial concern for the beginning or the end, but, throughout the period of illumination, steadily and rhythmically discharges impulses, at a faster rate in strong light, a slower one with feeble illumination. And finally, what seems at first most bizarre of all, there exists one kind of receptor which remains entirely quiescent during the whole period of illumination but after its close begins to chug away as regularly and steadily as did its fellow while the light was on. Such a cell, then, discharges continuously in the dark and promptly stops with light. If the presence of light is a stimulus to the one type, the absence of it must be equally considered as the stimulus to the other.
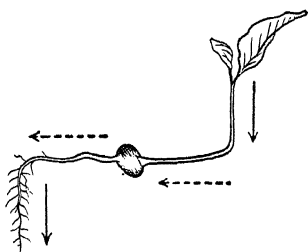
Fig. 101. Dotted arrows show the direction of the pull of gravity during the first period of growth. The seedling was then turned so that gravity pulled in the direction of the solid arrows—and the root and stem bent accordingly.
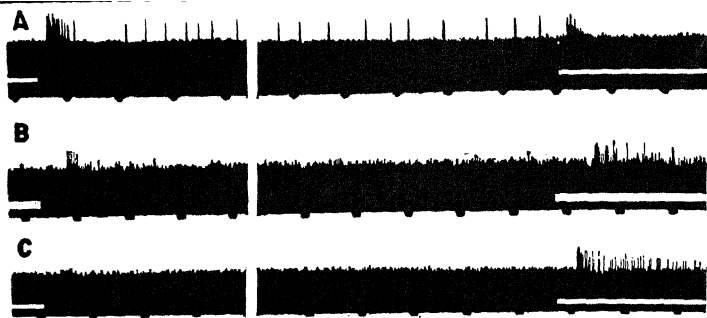
FIG. 102. Photograph of action potential spikes in nerve fibres, set up from various type of receptors in the frog's retina. Light on eye blackens out the horizontal white signal line. Humps at bottom are 1/5th second apart. Receptors in A discharge during illumination; those in B, when light is turned on or off but not during steady illumination; those in C become active only when the light is turned off. (Courtesy of H. K. Hartline.)

But the story is not quite ended. Besides the ability to respond to an environmental change, the irritability present in all living structures, there is another universal attribute, the tendency progressively to discount or ignore this change, called adaptation. Of this we are well enough aware from our daily experience. The water feels cold when we first enter it but then becomes thermally neutral, and when we finally emerge again, the air, which originally was neutral and will soon be so again, seems for a while distinctly warm. We feel at first loaded down with a pack on our back, then become unaware of it, and feel amazingly light and buoyant after it is removed. Over even longer times such adaptation is present. If, during the cold months, the temperature reaches 60° on some sunny day, it feels positively hot, while a similar day breaking the summer heat is distinctly chilly. A wedding ring is felt as an excrescence on the finger for days or weeks after it is placed, but eventually it becomes so completely a part of the body that its temporary absence is continuously and unpleasantly in consciousness. Were it not for this adaptation, shifting a ring to a different finger would not be the excellent memory-jogger that it is.

Every stimulus applied to a cell, therefore, initiates two kinds

of change. On the one hand, it disturbs the metabolic and structural equilibrium in a manner tending to produce a response; on the other, it initiates changes, less understood, which oppose these disturbances and act to restore the resting state despite a maintained alteration of the environment. If adaptation is very quick, the stimulus may lead to a single response or to none at all. If it is slower, a few or many responses may result. If the response occurs within a thousandth of a second after the stimulus begins, whereas adaptation requires several minutes, many thousands of responses may result. Over a short time interval these will seem regular and appear to be at constant intervals, but when examined over a longer time range the interval is seen to become slowly longer, as adaptation increases, until finally it becomes infinite as responses
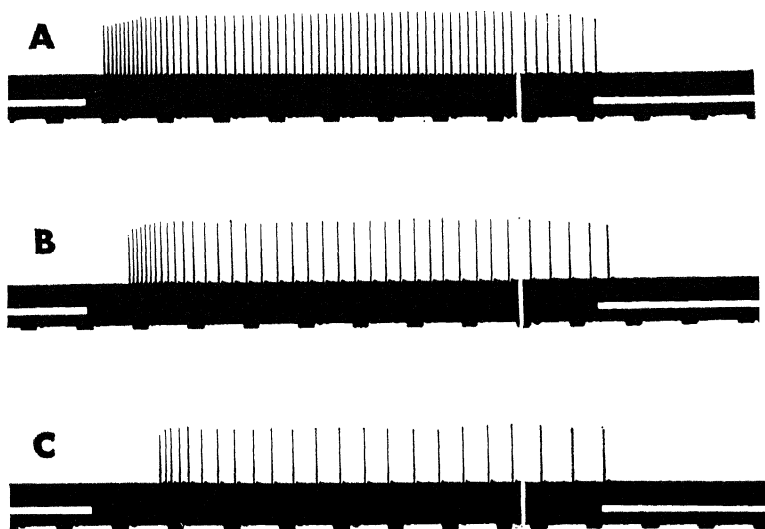
Fig. 103. Photograph of action potential spikes in a single nerve fibre, set up from a single receptor in the king crab's eye. Light on the eye blackens out the white signal line. Humps at bottom are 1/5th second apart. The light was kept constant during each exposure but was most intense in A, ten times weaker in B, and ten times weaker yet in C. Note that nerve discharges start sooner and keep a higher frequency, though they are not larger individually, with strong light; but in all cases they show a slowing after the start. (Courtesy of H. K. Hartline.)

cease. Finally, if adaptation is practically absent, then the cell
will continue its regular rhythmic activity so long as the exter-
nal conditions which are necessary to it are maintained. The
geotropic response of the growing root shows no adaptation; the
nerve fibre giving a single impulse when a constant current
is started shows an extremely rapid and complete adaptation;
and the nerve fibre with low calcium, or one normal type of
retinal cell, shows very slight adaptation and continues to dis-
charge with but little diminution of rate as the stimulus con-
tinues to act upon it during minutes.

Now what of the heart or nerve cells which beat continuously
only when the usual amount of calcium is present; of muscle,
which does so only when calcium is decreased; or of these
retinal cells which discharge regularly only in the dark? How
does a continuous presence or absence of calcium or of light
or of what one will, determine the occurrence or the loss of
rhythmical activity? Paradoxically enough, regular rhythmic
action can occur only as a result of constant and unvarying
forces. The steady swing of the pendulum depends on the
constant pull of gravity; a flag flaps away evenly only when
the wind is steady; the uniform diffusion of ions into a jelly
with which they react causes a precipitate to form in repeated
bands; and the continuous activity of some endocrine glands
underlies the rhythmic changes of ovulation and menstruation.
The steady cell metabolism offers, likewise, an understandable
mechanism for these cell rhythms.

Suppose, for example, that some cell goes into action, dis-
charges its function, when a particular substance within it or at
its surface reaches some critical concentration. Concretely,
though probably incorrectly, let us say a muscle cell contracts
when its creatine-phosphate content increases above some fixed
value. At rest and in metabolic equilibrium the concentration
of such a substance, S, remains the same, for the rate at which
it is being formed is equal to that at which it is being removed.
Creatine-phosphate, to keep our example, might be forming

from adenyl-pyrophosphate and creatine and in turn reacting with glucose to give hexose-phosphate and creatine. The concentration of S will increase, then, if either the rate of its formation is accelerated or that of its destruction retarded. When S reaches the critical concentration the cell will discharge and some of the substance be used up in the new reaction. After the single response, however, S will again accumulate, a second response will follow in time, and so on indefinitely. So long, then, as S is formed faster than it is destroyed the cell will manifest repeated and regular activity. The more rapidly S accumulates, the briefer will be the interval between successive responses and the faster the rhythm.

The rates of the two reactions concerning S are, however, dependent on the many factors, including environmental ones, which we have noted. If potassium ion enhances the formation of creatine-phosphate and calcium ion its destruction, as in
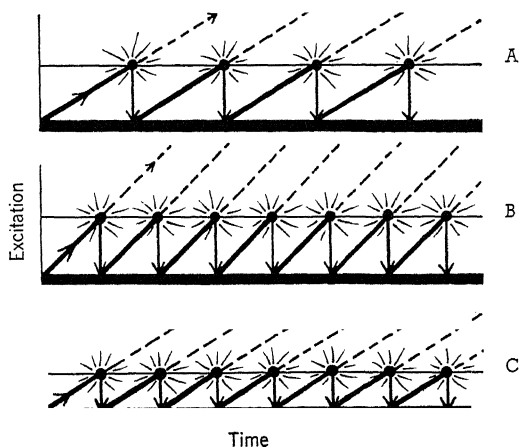
FIG. 104. Excitation increases at a constant rate until some threshold value is reached. Then the cell goes into action, gives a response, and excitation is decreased. A regular rhythm of repeated responses is the result (A). If the excitation increases more rapidly (stronger stimulation, B) or if the threshold is lower (more irritable cell, C), the frequency of the rhythm is increased.

fact they do, a potassium increase or a calcium decrease should cause this substance to accumulate in muscle cells and so initiate rhythmic contraction. This does occur. If the formation of S in some retinal cells and its destruction in others be increased when the cells are illuminated, then the one group should continue to discharge in the presence of light, the other in its absence. There is no greater mystery, then, about the stimulation produced by a decrease of some particular substance or energy form than there is about that due to an increase.

The more difficult problem, and one all too vaguely understood, is that of adaptation, the failure of responses to continue indefinitely. For the retinal cell that discharges only once or a few times in a steady light, it must be assumed not merely that the formation of S is accelerated by light (or its destruction slowed) but also that some other change renders this effect only temporary. Either the accelerating influence of light rapidly peters out, presumably because of other chemical reactions, or else a concentration of S which initially suffices to discharge the cell machinery becomes later insufficient. The alteration in physico-chemical structure of the cell with activity must be assumed to have raised its threshold so that a still higher concentration of S is needed to excite.

In any event, the activity of a cell does change its capacity for further activity. Over very short times, function commonly improves with functioning, the warming-up process; over longer times it is diminished, fatigue; but some positive effect remains behind which is cumulative over still longer intervals, training. Thus, as exemplified in muscle contraction and coordination, performance improves with activity through the warming-up period, falls off more slowly with advancing fatigue, and, on repetition over days and months, steadily improves as the athlete becomes practiced and trained. Similarly, with use the soles of the feet grow thick though those of the shoes grow thin. The metabolic basis of adaptation can at

present be only guessed at; though it is probably closely related to such phenomena as growth, learning, and evolution, which resemble it on greatly extended time scales.

Specialization for Action

One universal attribute of protoplasm is irritability, for sufficiently intense environmental changes of almost any kind will change its state of activity. This must be true of protoplasm as of any complex system in quasi-equilibrium and not completely insulated from its surroundings. Actually, complete insulation never exists; and for living organisms the interchange between body and environment is intimate and continuous, which makes them peculiarly susceptible to the condition of their surroundings. Even the most primitive cells respond to applied light, heat, chemicals, and the like by a change in metabolism or growth or by actual death when conditions become sufficiently extreme. If the cells have means of locomotion they will move from a region of excess light or acidity or, failing this solution of the problem, they may at least become metabolically dormant and encase themselves in a layer of resistant and impervious material. With increasing complexity of the cell and differentiation of its parts for special activities, still more with the organization of many cells into a single social unit so that whole cells or groups of them may differentiate and specialize, the problem of environmental adjustment has been met ever more efficiently.

All parts of protoplasm do not remain equally responsive to stimuli, but certain localized regions or cells may become especially sensitive, each to some particular physical or chemical agent. Protozoa with a tiny photosensitive pigment spot are more reactive to light than are others which lack it; they are able to respond to lower light intensities than can undifferentiated protoplasm and so, for example, may retreat from harmful illumination before the cell is damaged. The highest

efficiency in photoreception is attained by cells in the verte-
brate retina which are sensitive to light changes millions of
times smaller than those required to act upon unspecialized
protoplasm. In like fashion, cells in the ear are excited by
mechanical vibrations quite beyond the range of other cells;
receptor endings in the skin, reinforced often by the lever
action of hairs, are acutely sensitive to touch; and cells of the
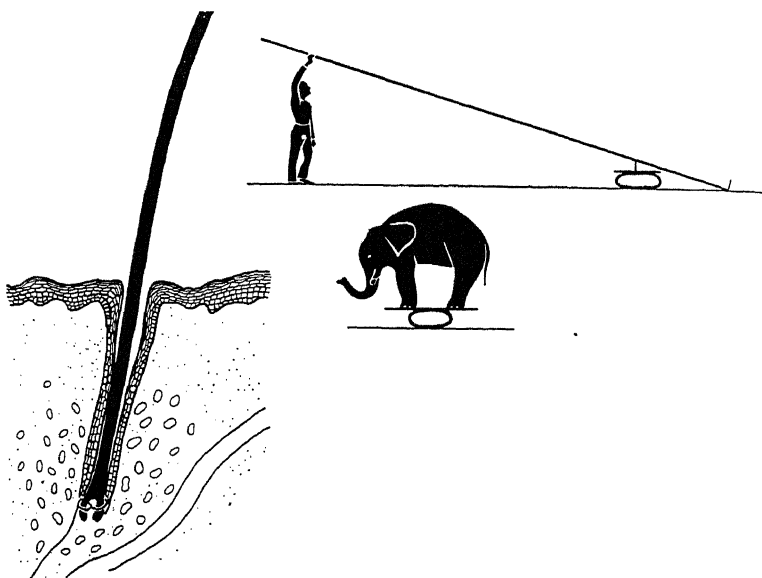
FIG. 105. Touch receptors in the skin are exquisitely sensitive to direct
pressure; and when they are located at the base of a hair which acts like a
lever upon them—as in the diagram of the man squeezing the ball—an ex-
tremely light touch to the hair easily evokes nerve impulses.

mouth and especially the nose are unbelievably susceptible to
chemical stimulation.

It has been shown that a sex gland of one single female moth
exposed on a still night will, by odor, attract males of that
species from distances up to three miles! No chemical tests
have been devised by man which will detect particular sub-
stances in dilutions anywhere approaching those to which these

chemical receptor cells respond. No combination of lenses and supersensitive plates begins to compete with the constant performance of the eye. Until this century, a frog nerve was often used to detect feeble electric currents, for none of the physical instruments that had been invented were so sensitive as it to electric flow.

Specialization permitted an equally impressive achievement in elaborating the actions of which protoplasm is capable. The slow, clumsy, and uncertain movements of the amœba have been improved by the appearance of cilia and later of special motile cells. These culminate in striated muscle, able to contract and relax several thousand times a second in some insects, and to exert a strong directed pull. Compared to the confused chemical activity of unspecialized protoplasm, the expert manufacture of particular substances by gland cells is a baker's "creation" versus hash. The feeble potentials accompanying all protoplasmic activity have been so cunningly elaborated and superimposed upon one another in the electric organ of the torpedo that a shock of some hundreds of volts can be delivered in attack or defense. These special effector cells and organs have each acquired a unique expertness in behavior just as the receptor cells have acquired their individual exquisite sensibilities.

And one other important development has been necessitated by this division of labor between spatially separated regions; the information received by the receptor must somehow be passed on to the effector. So the simple and casual transmission of excitation along the protoplasmic surfaces of single cells has been specialized into the highly elaborate and fast nerve impulse shooting along special nerve paths between all parts of the large mammalian body. Further, with increasing numbers of units, the problem soon passed from one of connection to one of coordination, and mere connecting pathways no longer sufficed. Stations for adjustment, coordination, and integration appeared, developed into the nervous system, and

culminated in that most amazing of all manifestations of protoplasm's potentialities, the brain of man.

But this path leads away from the general problems of cell activity on which we have set our feet. Grossly or delicately, as raw protoplasm or elaborate organisms, all living things are sensitive to their environment. As it alters so do they respond, and nearly always in such a manner as to maintain their integrity. In this sense, organisms adapt to their environment and behavior shows purpose.
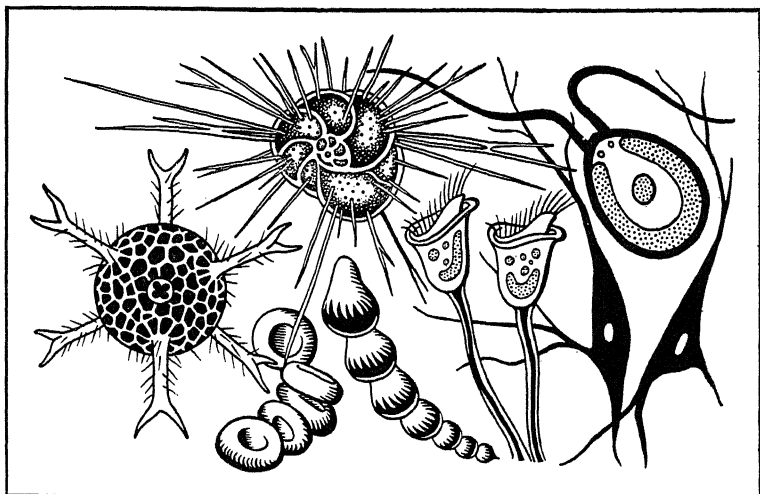
Chapter Nine: Through the Microscope—
Cell Structure

If the universe were a continuum, like thick pea soup, the idea of "unit" would probably never have come into existence and we should have no number system. Actually, units do exist—systems coherent in themselves and delineated from their surroundings sufficiently to be identified as such. Even the seeming continua have been resolved by advancing knowledge into discrete quanta: protons, atoms, and molecules of matter, electrons and photons of energy, etc. Single living things are obviously units, and in the smaller organisms no clear-cut sub-units occur; the cell is the organism. But as individuals of larger and larger size appear over the biological horizon, this is no longer true, for cell size does not increase beyond very modest dimensions and the bulk of the whole organism enlarges by a multiplication of cell units. Why should not the larger organisms simply increase all their proportions uniformly to become merely bigger single cells? Many answers to this question are available and possibly all play a role.

FRONT END

Clear outer layer
Cilium
Stiff outer covering
Trichocysts
Contractile vacuole
Small nucleus
Large nucleus
Top view of surface cilia and network of coordinating fibrils
Food vacuole
Contractile vacuole
Cilia
Oral groove
Mouth pore
Gullet
Food vacuole
Anal pore

REAR END
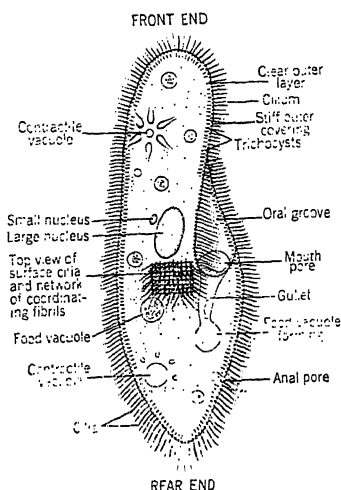
FIG. 106. Paramecium, with different organelles indicated in various portions. Many of these exist all over the tiny body.

One reason is tied up with the specialization of function we have just examined. With many different kinds of cells, each an expert in discharging its own particular duty, greater total efficiency is possible than when each cell must do everything itself. This argument would be more impressive if we were unaware of the existence of protozoa which, though single-celled, still show most elaborate specialization. Instead of devoting a separate cell to each functional need, some special part or organelle in the one cell serves the same purpose and apparently just as satisfactorily.

No, the cellular organization is not essential to a high degree of differentiation, though it does, of course, enhance this. Rather it seems a prerequisite to size as such and so recalls the geometrical truth that linear dimensions, surfaces, and volumes of solid objects alter their relative magnitudes as their absolute ones change. As a droplet of protoplasm increases in radius, its surface grows as a square, its volume as a cube. Since only the surface area is available for interchange of any sort between organism and environment, there must come a size at which the surface is no longer adequate to the needs of the more rapidly increasing volume of protoplasm. Probably similar problems exist between nucleus and cytoplasm, for in many cases cells divide when the ratio of cytoplasm to nucleus exceeds a definite magnitude. We cannot stop to examine the forces involved in mitosis—surface tension, osmotic flow, micellar shortening, electrostatic attraction and repulsion, and the like—

but the case of the whole cell is most clear from the chemical point of view.

Geometry and Speed ·

Metabolism occurs throughout the protoplasmic mass, but substances diffuse in and out only through the surface area. If any oxygen is to reach the center of the cell (since it is used up all along the way from the cell surface to the center by the respiration of the protoplasm) the external concentration of these molecules must exceed some critical value. This value is determined by the ease with which oxygen passes into the cell, the rate at which it is used per unit quantity of protoplasm, and cell dimensions. Respiration rate and diffusibility appear in simple proportionality in the equation which expresses these relations, but cell size, radius for a sphere or a cylinder and thickness for a plate, enters as the square. If,

AREA

RADIUS

VOLUME

FIG. 107. As a cell increases in radius, its volume increases much more than does its surface.

therefore, air (20 per cent oxygen) just supplies the center of a cell 0.1 mm. in diameter it would take pure oxygen (100 per cent) to supply an equivalent cell of diameter 0.23 mm. (Five times the oxygen permits $\sqrt{5}$ times the diameter.)

Obviously cell size could not increase very far without jamming the metabolism, for the surface always constitutes a

bottle-neck to chemical traffic. If cells forswore the spherical shape and, by flattening, folding, twisting, and sculpturing of various sorts, kept the surface increasing with the bulk, they could become large, of course; but it seems hardly necessary to consider the other limitations, if the mass of the human body were flattened into a pancake a fraction of a millimeter thick. Even a convoluted surface is far more easily achieved by the proper grouping of many small units than by any type of molding of a single large one; and the forces of capillarity, surface tension, adhesion, and the rest, also militate against a single large cell.

The same surface-volume problem reappears over and over again in the organization of more complex living beings. The absorbing surface of man's digestive system is greatly increased by having a tube over twenty feet long coiled about in the abdomen, by having the lining of this tube folded upon itself in a long series of accordion pleats, and by piling up the individual cell groups which cover each fold into an almost continuous series of microscopic fingers, like the pile of a bath towel. The surface in his lungs, through which oxygen is exchanged for carbon dioxide, is built in part of minute sheet-

like cells attached edge to edge to form a membrane, and would occupy roughly a tenth of an acre if stretched out flat. Actually it is ballooned out around the ends of the final hollow twigs of the bronchial tree in the form of myriad tiny grapeskins, so that this enormous surface is tucked away compactly in part of the chest volume.
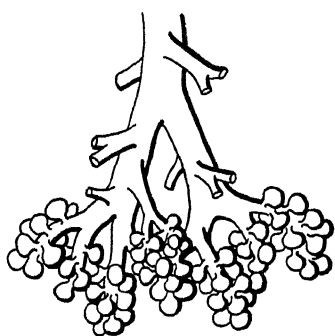
FIG. 108. The air sacs, clustered like grapes about the air tubes, give the lung an enormous surface in a small volume.

Similar hardly credible, but true, stories could be told about the surfaces of the blood vessels,
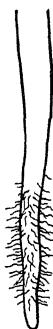
FIG. 109. "Hairs" near the tip of a growing root. Each one is an extension of a single cell.

those within the kidneys, in the reproductive organs, and so on. Even the brain, as it progressively grows in volume, simultaneously develops a wrinkled or convoluted surface, although in this case, since there is no particular passage of substances across it, the reasons for the surface increase are less obvious. Plants spread out many flat leaves and elaborately branching roots to gain surface and the cells constituting the outer layer near the root tip are themselves prolonged into tiny hairs for the same end. On the macroscopic scale, no less than on the microscopic colloidal one, surface area is a vital consideration; and just as division into colloidal particles increases this desirable attribute, so also does the division of organisms into cells.

Another group of facts may find meaning in terms of the rate of cell metabolism. It is found (with some striking exceptions) that the cells of a large animal are somewhat larger than those of a small one, especially within certain cell types, such as those of the nervous system. These are larger in the cat than in the rat and much larger in the elephant than in the mouse. Cell size does not vary in proportion to animal size, of course, but it does seem to follow in this case the two-fifths power of animal size. This relationship is certainly not directly explicable in terms of the total mass of the organism, for the problem of surface and volume is essentially one of the individual cell, and besides, greatly varying numbers of cells are present in different-sized animals.

Another fact is helpful here; the metabolic rate for cells of small animals is higher than that for the cells of larger ones. Gram for gram and ounce for ounce, the brain of a mouse uses most oxygen, that of a dog least; and occupying intermediate positions in order of size of the animal are the respiration rates for the brains of the rat, guinea pig, rabbit, and cat. The calcula-

tions concerning oxygen diffusion show that, for a given ex-
ternal oxygen concentration, the diameter of a cell must de-
crease as the respiration of its protoplasm increases, the size
changing as the square root of the respiratory rate. If proto-
plasm of small animals is geared at a higher metabolism than
that of large ones, this would well account for its being
organized into smaller cells. Why metabolism should vary
with animal size is not germane to the present discussion,
though itself a most interesting consequence of surface-volume
geometry.

Engineering Problems

Besides these metabolic or chemical considerations, a num-
ber of purely physical ones contribute to the cellular plan of
organization. For one thing, a large mass of viscous but fluid
protoplasm would certainly not possess sufficient rigidity to
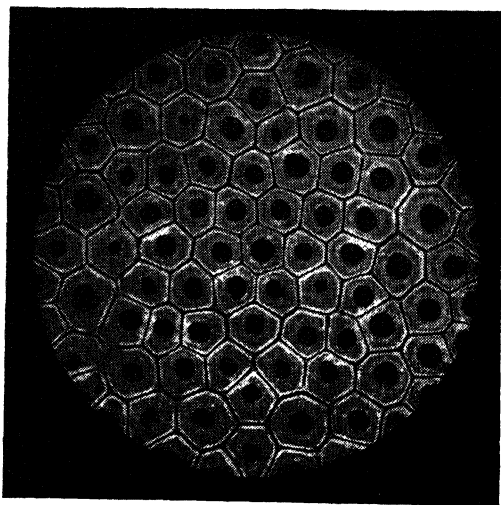
FIG. 110. Typical cell-like structures formed by drying and diffusion. (From
D'Arcy W. Thompson, *Growth and Form;* by courtesy of the author and of the
publisher, Cambridge University Press.)

maintain any particular form but, like a thick soup or a thin jelly, would gradually flow into whatever container existed. The same protoplasm, however, organized into units possessing elastic or semi-firm restraining walls, closely adherent to one another, can show a high degree of rigidity. In much the same way, egg albumen and water separately are fluid and formless; but beaten together, the separate water droplets each become surrounded by an albumen shell and the resulting meringue is a firm mass which will hold any shape impressed upon it.

FIG. 111. Animal cells (above, liver) have pliable walls and are motile but not strong. Plant cells (below, alga strand) have reinforced their walls with an outer layer of wood and become rigid but motionless.

Throughout the range of organisms there is a continuous compromise between problems of rigidity and of mobility. When cells have very rigid walls or are surrounded by inert walls of rigid material, such as the cellulose of wood, extreme strength is obtained but motion is entirely lost. This is the solution made by plants. Animals, on the contrary, have retained relatively weak and pliable walls to their cells and so preserved motility at the expense of structural strength. In many ways this proved a happier solution than that of the plants, since the higher groups of animals, at least, have gained rigidity at the tissue level rather than the cell level by introducing special skeletal structures. The great group of insects, arthropods, covered the body surface with tough chitin to which muscles were attached on the inside and which were so compounded with flexible joints as to permit considerable movement. The vertebrates introduced first cartilage and then bone, which is still further toughened by the deposition of lime and related salts. Their very mobile and plastic muscles are attached

to these bony surfaces and extend between two rigid bones able
to move upon one another by virtue of well-lubricated joints.
On a smaller scale, within the vertebrates, appear both solu-
tions of this same dilemma. Some animals have become heavily
armored externally and slow and awkward in motion, others
have forsworn mechanical protection in favor of speed and
agility of movement. One need not go further than the hare
and the tortoise, for this fabled pair illustrates the point
admirably.

Another matter that is often lost sight of is that accurately
enlarging a given animal, point for point, still changes many
relations. Writers of imaginative fiction delight in portraying
Martian or other undiscovered men as identical with ourselves
except for a gargantuan size, two to ten times the normal five
foot seven of human stature. But geometry still exacts its pound
of flesh for every size increase. If all anatomical proportions
remained the same, a doubling of height would mean eightfold
increase in weight with only a fourfold enlargement of the
cross section of the leg bones which must support it. Sooner or
later something would give. It is not surprising that, as animals
increase in size from one species or genus to another, profound
changes in body architecture regularly occur. Legs increase
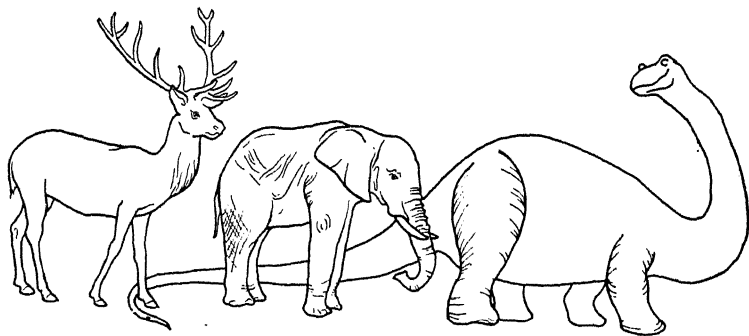
Fig. 112.   When a deer, elephant, and brontosaurus are reduced to the same
height their legs do not reduce to the same diameter. The larger and heavier
body requires for its support legs which are more than proportionally stout.

in girth more than in length, special buttresses sprout out of the bone and, in fact, good general engineering practice is adhered to. The kitten's tail stands as a straight spike, but that of the cat droops at the tip—it is suggested that the larger member, with only a proportional increase in dimensions, cannot fully carry its weight. Large animals have shorter legs in proportion to size than small ones. Actually, the largest animals that have ever existed, some of the wading dinosaurs and the present-day whales, could not support themselves in air, since the materials available for animal structures would give under their weight. Such huge size and mass are possible only when the powerful buoying action of water is utilized.

When we turn from the relatively simple problems of sheer weight and static structure to those of movement, the difficulties multiply. Inertia depends not only on mass but on lever arm. A rod held at one end and given a lateral jerk, still better an angular one, will break near its point of support if the movement is sufficiently abrupt. The angular acceleration required to break a large rod is less than that needed to break a smaller model; for strength varies as cross section, a square, while mass varies as a cube and the lever arm adds still another linear factor. With increased limb size, consequently, must go a progressive slowing of movement. Possibly here is another clue to the lower metabolism per unit mass of large animals as compared to small. Certainly it is true that, measured against their own sizes and often even in absolute velocities, small animals move far more rapidly than do large ones. The elephant's heart beats at a rate of 25 times a minute, that of the mouse at 500. A trained musician can contract and relax the muscles moving his fingers at about ten times a second, the hummingbird can flap its wings 500 times a second; and the bee, still more the mosquito (witness the high-pitched musical note produced by its vibrating wings), can do so several thousand times in the same interval.

One more sample of the problems encountered on magnify-

ing organisms may suffice to relegate the Martian giants irrevocably to the limbo of the fanciful. The individual cell buried somewhere in the mass of its fellows does not, of course, depend upon substances reaching it by diffusion from the surface of the whole animal or even from particular absorbing organs. Materials must be delivered directly to its surface far more rapidly than could be achieved by this slow physical process. Some sort of extracellular fluid must continuously be moved past the cells, bringing new supplies and removing wastes.

In the vertebrates, a special closed circulatory system of hollow conducting tubes has been developed to meet this need. The circulating blood is pumped by the heart into large arteries which progressively branch into smaller and more numerous ones which finally empty into extremely numerous, narrow, and short capillaries. From these the blood passes in reverse manner from twig to branch to limb in the venous channels until the largest veins disgorge it again into the heart. The walls of arteries and veins, even their small divisions, are relatively thick with layers of muscle, elastic, and fibrous tissue to give them strength and some degree of contractility. The blood rushes through them rapidly and little or no exchange between blood and tissue cells can occur.

Within the capillaries, however, the blood moves relatively very slowly; the walls are extremely thin, composed of a single layer of flattened cells; and the surface is great, because there are many vessels of minute diameter. Capillaries are so numerous throughout all tissues and organs and so well distributed that, with few exceptions, every tissue cell in the body is in the close vicinity of one, certainly not more than one or two cell diameters distant. It is at the capillaries, then, that an exchange of substances occurs between the blood and the tissue fluid which bathes the cells themselves. The capillary wall must be permeable to water and salts, to the simple food substances, such as glucose and amino acids, to the waste products of cell metabolism, and in general to simple dissolved mole-

cules and ions. The fluid part of the blood, or blood plasma, contains, in addition, colloidal proteins which do not normally penetrate the capillary wall.

And now the engineering problem presents itself. The blood in arteries is at a fairly high pressure. This falls rather sharply in the capillary bed, because of the high frictional resistance to flow, and continues to fall slowly along the veins and back to the heart. If blood is to flow from capillaries into veins, as of course it must, the pressure in the capillaries must always be higher than that in the veins. Now consider a capillary in a man's foot, which empties its blood into veins that run vertically some four feet to the level of the heart. This column of fluid, by sheer hydrostatic weight due to gravity, must exert a back-pressure in the capillaries of four feet of water or about 40 millimeters of mercury. But such a fluid pressure inside the capillaries, not balanced by an equivalent pressure outside, would tend to filter or squeeze the fluid portion of the blood out of the capillaries into the potential spaces between the cells. A steady seepage of water into the tissues, however, would soon swell and water-log them and produce the condition known as dropsy or edema. Clearly some other force is acting to balance the hydraulic pressure and either to draw water back into the capillaries or to prevent its leaking out.

The source of this force becomes clear enough on recalling that the capillary walls are impermeable to the plasma colloids. The colloidal particles, held within a semi-permeable membrane, exert an osmotic pressure tending to draw water in toward the colloidal suspension. This osmotic force is proportional to the number of dissolved particles and, for the 7 per cent of protein in plasma, turns out to be nearly 40 mm. of mercury pressure. Certainly it is no coincidence, but another example of the beautiful balance found whenever living things are studied, that the hydrostatic pressure tending to force water out of the capillaries is nicely balanced by an osmotic pressure tending to draw it in.
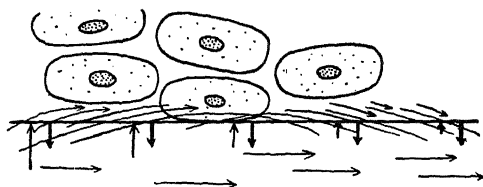
Fig. 113. The "colloid osmotic pressure," tending to draw water into a capillary, is of equal magnitude over its whole length. But the simple physical blood pressure, tending to filter water out, is greater at the arterial end, less at the venous end. Water therefore does filter from the blood plasma into the intercellular spaces at one end and most of it is reabsorbed at the other. This flow of "tissue lymph" past the cells helps to nourish them more efficiently.

Actually the situation is a little more complicated, for the blood pressure is higher at the entrance to the capillary and lower at its exit. Some fluid does, therefore, leave at the beginning of the vessel largely to return at its termination after percolating along between the adjacent cells. Also, the veins in the legs have valves which permit the blood to flow only toward the heart and, as the muscles in which these vessels are embedded alternately contract and relax, the veins are irregularly squeezed upon and the blood in them milked upward. This helps reduce the hydrostatic back-pressure on the capillaries and, indeed, failing this muscular assistance, the pressure balance in the capillaries is slightly disturbed. It is for this reason that prolonged standing, during which there is little rhythmic muscular contraction, tends to produce a swelling of the foot and ankle. This is very easily demonstrated in a home experiment. Remove one shoe and stand on a low foot stool or a stair with the shod foot, allowing the other to hang quite limply in space. After a few minutes it will prove impossible to replace the shoe, because of a mild (and temporary) edema.

The problem of increasing animal size is now clear enough. If the height from ground to heart is doubled the hydrostatic back-pressure on the capillaries of the foot will require 80 mm.

of protein osmotic pressure to offset it, and so 14 instead of 7 per cent protein in the blood plasma. Such a high protein concentration, however, offers new and even more serious problems. As the amount of colloid in the plasma water increases, viscosity mounts rapidly. With any considerable protein increase, blood viscosity would be so great that the heart would be quite unable to pump the blood through the capillary bed. A certain amount of leeway is, of course, present. Another solution would be to add auxiliary pumping stations along the way, which would permit a great increase in an animal's height without gumming up its blood or afflicting it with edema. Scientists have long yearned to study the giraffe, in view of these problems, to find out how it successfully maintains a circulation in which blood travels a vertical distance of over fifteen feet from hoof to head and perhaps half this from hoof to heart. But in any event, these considerations suffice to show the utter impossibility of applying to a large organism the same structural and functional solutions which have satisfactorily met the problems of a small one.

Returning from this excursion around the whole multicellular organism to the cell itself, we find mechanical forces again playing an important role. Surface tension depends on the attraction between molecules which tends to draw them together. The actual pull exerted is determined by the kind of molecules in a surface or film and by the surface curvature. If two different-sized bubbles are blown from the same soap solution, the pressure in the smaller one is greater than that in the larger. This is easily shown by connecting the
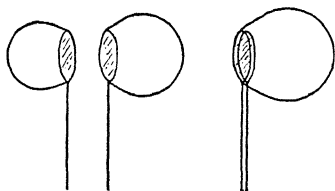
Fig 114. Two soap bubbles, of the same liquid but of different sizes, will not exert the same pressure on the air inside. The small bubble, with the greater curvature, has the greater pressure; if the two are joined and the partitioning membrane is removed, the small bubble squeezes all its air into the larger one.
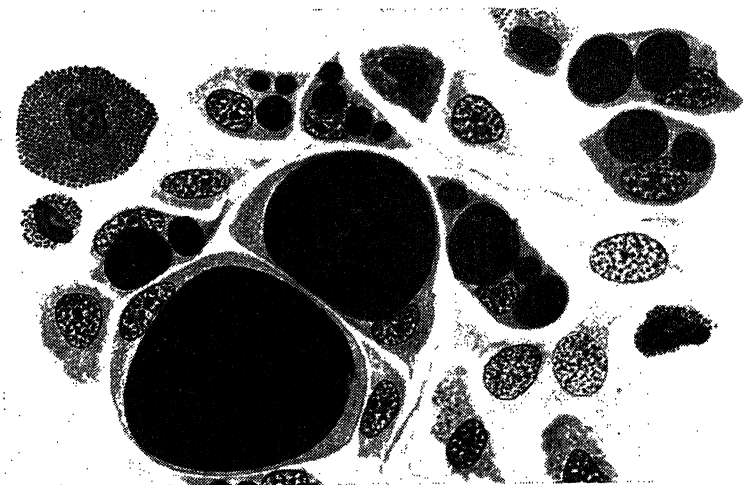
FIG. 115. Young (small) fat cells with many fine fat droplets and old cells with few or a single large one. Fat is stained a solid black. (From Maximow and Bloom's *Textbook of Histology*. Copyright W. B. Saunders Company.)

two with an open cylinder, for the small one then rapidly collapses to a flat film and forces its contents into the larger, which becomes still more distended. Since both membranes are made of like molecules, the greater force exerted by the small bubble is a result of its greater curvature. The same relations hold for membranes surrounding fluid masses, so that small drops tend to coalesce to form larger ones and thereby reduce the surface energy. This is nicely illustrated in the developing fat cell which forms many tiny fat droplets in its cytoplasm, only to have them unite into a single large drop which fills most of the mature unit.

Suppose, however, that we are dealing with a chemically reactive system, as is a metabolizing cell, and that substances which tend to alter surface tension are produced throughout the mass and diffuse out through the surface. There may result a balance of conditions tending on the one hand to increase drop size and on the other to decrease it. Actually this whole

situation has been carefully explored mathematically and some striking relations established. A drop of substance A is suspended in water containing B and C which slowly diffuse into the drop and there react to form more A. The substance A also dissolves very slowly in water. It is then true that the drop will grow in size most rapidly at first, then ever more slowly until an "adult" size is reached, closely duplicating the growth curve of a cell or organism. Further, if certain reasonable surface tensions are assumed for these substances, on reaching some critical size determined by reaction rates, the drop will pinch in two to form paired daughter drops.

But this, of course, is the essence of cell division, and again comes the strong suggestion that the maximum size any type of cell can obtain is determined by its own metabolic rate. Differences in membrane composition and in metabolic products would still allow differences in size between various kinds of cells. At least this further evidence is interesting. Cell size is quite definitely related to nuclear size in any particular kind of cell. In various ways, the mass of nuclear material (chromatin) can be abnormally increased and decreased; and the cytoplasm then adjusts its volume to retain the proper ratio of the two. But the nucleus is rather particularly the source of oxidizing and other cell enzymes, so again size and metabolism are correlated.

Size and Shape

What is the actual range of cell sizes? And what of cell shape and the dimensions of non-spherical cells? The old problem of category boundaries recurs—when can we properly speak of a single cell and when have we transgressed any legitimate use of this concept? One biological criterion of the cell is that it is a single unit and cannot survive any serious fragmentation. Any one portion removed from the rest will shortly die. It is this criterion which establishes the neurone as a single

FIG. 116. Microphotograph of neurones in cerebrum, blackened by staining with silver salts and "developing." Low magnification.

cell. The nerve cell is composed of a roundish or pyramidal cell body some 0.1 mm. in diameter plus extensions or processes of varying length and number. Some of these processes extend out to form the peripheral nerves and, in man, a single one may be over a meter long. The fibre's diameter is considerably smaller than that of the cell body, usually less than 0.01 mm., yet the overall length of such a single cell is prodigious. If this nerve fibre is cut at any point, the part severed from the cell body dies and degenerates; hence its inclusion as part of a single nerve cell. A neurone, then, might be assigned the
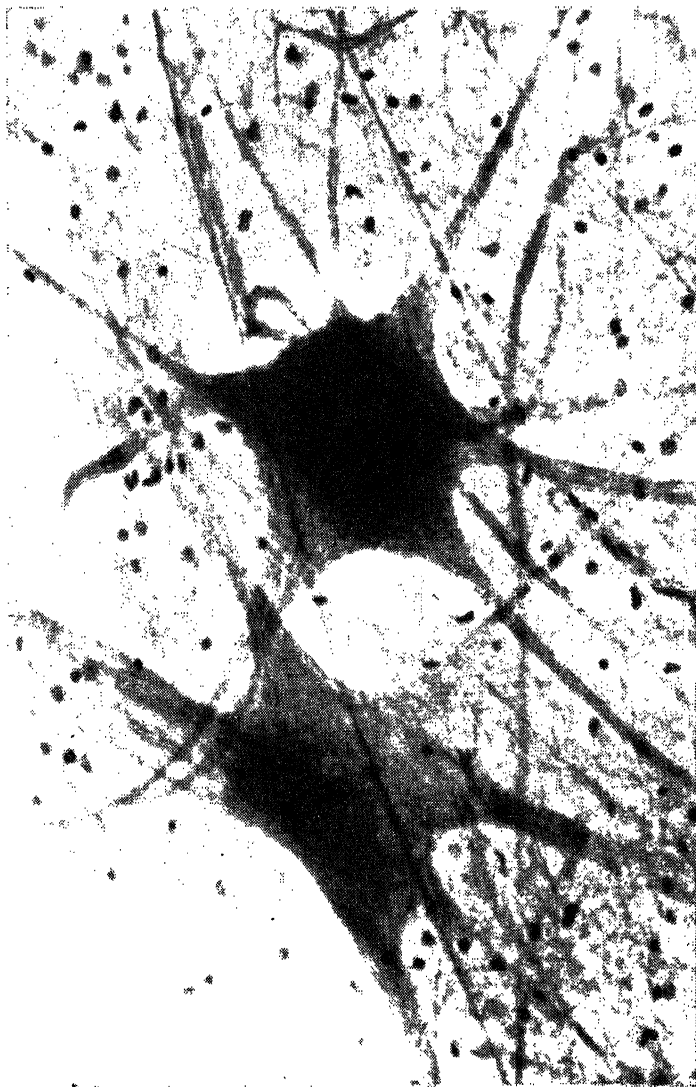
FIG. 117. Microphotograph of two motor neurones from the spinal cord, stained with dyes. Moderate magnification (nearly 200 times). The small spots are nuclei of ordinary cells.

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dimension of one meter or of 0.1 mm., depending on the point of view. For comparison with other reasonably spherical cells, the cell body size rather than fibre length must be used, in which case the neurone becomes a rather, but not extremely, large cell.

The largest cells are the unfertilized eggs, reaching their climax in great bird or dinosaur eggs well up to grapefruit size. These, like their more common relative, the hen's egg, contain a single cell plus a considerable amount of extracellular protein, the egg albumen. Even within the membrane surrounding the yolk, this huge bulk of lipoid material, like the albumen outside of it, is entirely inert stored food; and the cell concept must be severely stretched to include the whole yolk as a single cell. The living protoplasm which, becoming active after fertilization, divides and grows into the embryo is hardly more than a microscopic blob at one surface of the yolk. As well call a sack of flour containing a single meal worm one organism as label the whole stored food supply within an egg shell containing a speck of protoplasm a single cell. But if the case is fairly clear here it is far less so with smaller eggs, such as that of the frog, two or three millimeters in diameter. In these, though much of the mass is stored nourishment, it is not clearly segregated from the active protoplasm; but when division starts the inert pole of the egg lags behind. Finally, many marine eggs, up to several tenths of a millimeter in diameter, have their reserves distributed throughout the cytoplasm and the entire mass divides evenly when development is initiated. Even with these, 95 per cent of their cytoplasm can be amputated and the remaining fragment containing the nucleus will regrow a whole healthy cell.

Another difficulty appears in such a structure as the heart. This is built of branching and interlacing muscle cells, each some 0.01 mm. in diameter and maybe 0.2 mm. in length. However, these muscle cells are not separate but, on the long axis, smoothly pass into one another. That the individual muscle
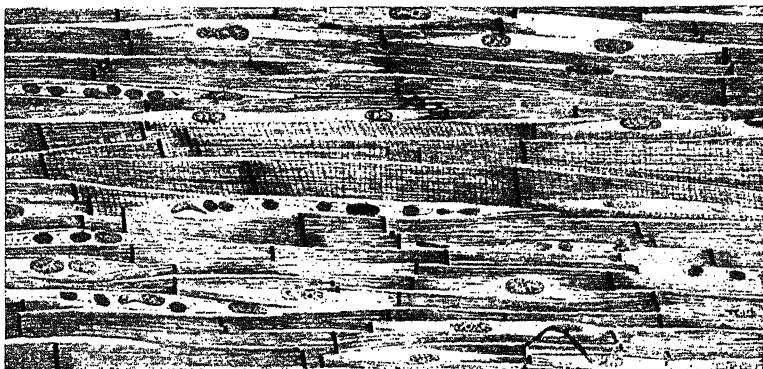
FIG. 118. Microphotograph of heart muscle. (From Maximow and Bloom' *Textbook of Histology.* Copyright W. B. Saunders Company.)

cylinders are really a continuum is dramatically proven by watching a minute protozoan parasite swim freely in the fluid protoplasm from one to another. One uninterrupted cell membrane invests the entire branching mass and an excitation applied at any one point ripples along this membrane in all directions, so that the entire heart contracts as a unit. Yet by other criteria the whole heart is not a single huge cell but rather a fusion, or syncytium, of millions of individual cells which have only partially lost their separate identities. For one thing, the myriad nuclei of these many cells are still scattered about in the cytoplasmic continuum; for another, separating these muscle bundles from one another does not entail their death; and finally, as the heart forms, during embryological development, the cells are completely separate and only later do they partially fuse.

The upper size limit, then, for single spherical cells would seem to be somewhere under a millimeter. At the other end of the scale are, for example: leucocytes, about 0.01 mm. in diameter; bacteria, 0.001 mm.; and on down through viruses and bacteriophages into the ultramicroscopic shadows. But again returns the old problem of whether these more minute units can fairly be regarded as cells or, for that matter, as living

entities. Possibly a safe lower range to set would be a tenth of a micron or 0.0001 mm. (A cell of this size would contain few more than 1,000 protein molecules.) Here then is the total range of the cell spectrum, from 0.0001 to somewhere under 1.0 mm. in diameter. If, indeed, cell size is ultimately determined by metabolic rate this is a surprisingly small variation, for diameter varies as the square of metabolism. A range of one hundred fold in metabolic rate should, then, exist between the most active and inactive cells; and the actually measured values do, in fact, spread over such a scale. It would be most desirable to know the values for metabolism of single-celled organisms, from micrococcus progrediens, $2/10^{12}$ cubic millimeter in volume, to opalina, 0.04; but such data are still very fragmentary.

Now what of cell shape? A drop of viscous protoplasm, surrounded by a membrane possessing some surface tension, must tend to round up into a sphere. This is the shape of many free-floating cells, such as eggs. When masses of such globules were packed together they would become faceted, their surfaces of contact being flattened out. Such irregularly flattened spheres are, in fact, found in many of the solid organs, such as the

FIG. 119. Spindle-shaped cells of smooth muscle. (From Maximow and Bloom's *Textbook of Histology.* Copyright W. B. Saunders Company.)

liver. If cells were packed in a single flattened layer, they would be formed into irregular prisms; if in a hollow tube, into irregular pyramids. These are the characteristic cell shapes in surface layers of body or organ cavities, as the loosely folded epithelium lining the stomach and intestine, and the fingerlike one lining tubules in glands. Cells that were originally fairly tall prisms would become thin flattened discs if the membrane which they composed were greatly stretched. Such stretchings do occur in the course of development, though not enough to account for all the membranes of thin endothelial cells. Cells subjected to stretch in one dimension would tend to a spindle or cylindrical shape, and all in the group would have their long axes more or less parallel. Such cells are found in muscle, especially the spindle-shaped ones of the smooth type, and in some of the connective tissues.

In fact, the orientation of cells and even of their contents under tensile or compressional stress is sometimes remarkable. The relatively few bone cells in the spongy interior of a long bone are not scattered at random but are grouped to form interlacing strands of bone. These sheets follow most precisely the lines of actual stress. Indeed they are deposited and located under the influence of these forces; for, when a broken bone heals in some distorted manner, the stress lines are shifted and the bony trabeculae soon rearrange and increase to occupy the new, mechanically correct, positions.

The micelles, neatly oriented along the muscle fibre, similarly have not attained their orderly and effective arrangement by chance. They are lined up under tension, and in the direction along which it is applied, just as are the protein molecules in natural silk or the modified cellulose ones in artificial silk. When a muscle is left for some time under no stretch, by cutting its nerve and so paralyzing it or by severing its tendon so that it exerts no pull when it contracts, then its micelles also lose their orientation. The dark and light discs soon blur together, the mass diminishes, and the shortening on stimulation becomes feeble, slow and, finally, is lost entirely. But

the consideration of how this comes about must be postponed.

These various cell shapes have been described as if arising solely under the influence of mechanical forces. This is surely not the whole truth and possibly only a small part of it, though it does account for many observations. Some cells, however, do not conform to such a physical picture. Not all single bac-
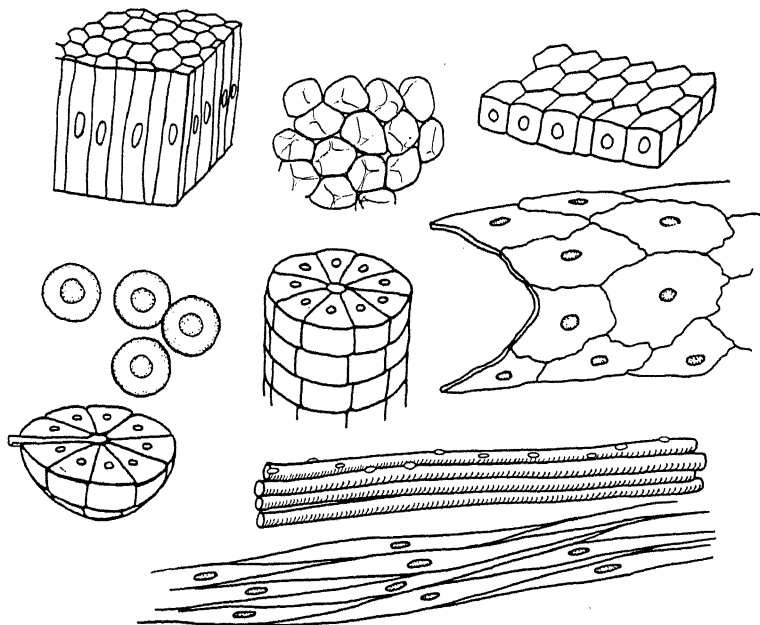
FIG. 120. Cell shapes conform to the structure and function of the tissues they constitute. At the upper left are columnar cells packed as a covering layer, or epithelium; and at the upper right are cuboidal cells forming a flatter epithelium. In the upper center are spherical cells squeezed into irregular faceted solids by being packed in an organ like the liver. Cells are stretched into thin plates (center row, right) to form the inner lining, the endothelium, of blood vessels and other hollow viscera; they are molded into prisms or pyramids to form test-tube-like glands of the stomach (center) or spherical glands (acini) of the pancreas (lower left). The free-floating blood cells (center row left) have smoothly curved surfaces with no edges of compression. The cylindrical cell masses of striped muscle and the spindle-shaped cells of smooth muscle (lower right) are elongated in the direction of tension exerted by the muscle. (The various cells are not diagrammed to the same scale.)

teria are spherical; some are rods or commas or spirals; single protozoa exist in almost every conceivable regular and irregular configuration; the enormous prolongations of neurones are certainly not to be understood in terms of mechanics. In such cases it is necessary to assume a rigid or semi-rigid structure within the cell, a sort of cell skeleton, which determines its form. The characteristic shape is, then, a result of determined growth and differentiation along specific lines, which is also a problem for future consideration.

Even here, however, cell shape commonly follows the law of minimal surfaces, its margin being shrunk about the framework by the ever-present tension at its surface. The forms assumed by microscopic organisms, and by macroscopic ones, often are in rigorous agreement with the outlines dictated by the mathematics and physics of surfaces, just as are those assumed by soap films. The radius of a spherical membrane which must cap each end of a cylindrical one can be precisely calculated, and just this value is found for the curved free end of cylindrical plant cells. On the other hand, when the cylinder, encased in a rigid woody wall, is partitioned by cell division, the new wall should, and does, run as a plane perpendicular to the long axis. The new surface forming to divide a conical or oval mass, however, cannot be a plane, if surface tension determines it, but must be curved like the surface of a lens. This also is regularly found to be the case—for single cells and for large shells. Snail shells, as a group, exhibit the most beautiful regularity and mathematical symmetry (that of the logarithmic spiral), for they are laid down under regular and repeated conditions of growth of the organism.

Such relations are best studied when cells solidify their outlines in wood or shell, but as each new surface forms it is at first fluid. These pliable margins are not always rounded up into balls even when no framework holds them taut, as witness the amoeba. But in this case the continuous metabolism is changing surface tension here and there, as air drafts cause a

flame to flicker. When the animal becomes dormant and metabolism falls to low ebb, surface inequalities disappear and the amoeba also rolls up into a sphere.

Making Structures Visible

Each tiny microcosm of the cell is teeming with visible and invisible structures. We have already paid some attention to its ultramicroscopic organization of ions, molecules, colloidal particles, micelles, and the like, and must now pay similar attention to the grosser, but no less important, structural patterns. Our description of these is likely to smack of the picturesque inaccuracies with which the blind men described an elephant, unless we look first at the methods of study. Of first importance, of course, is the microscope itself, able to magnify objects two thousand diameters or more and so extend the range of vision to dimensions of one ten-thousandth of a millimeter or less. Here is one of the fascinating chapters of scientific history; for what explorer has ever entered such a completely unknown part of the universe, peopled with such bizarre and unimagined beings, as · did the Dutch janitor a century and a half ago who ground and looked through powerful lenses!

But mere magnification is not enough; light will not penetrate thick cell masses. The obvious solution was to shave tissues into sufficiently thin slices so that they could be seen through, slices a few thousandths of a millimeter thick. Can soft sticky masses of protoplasm and cells be cut to such a thinness? Every paterfamilias knows the difficulty of cutting thin slices of meat that is nearly raw. The problem is easier with well-cooked meat in which, among other changes, the proteins have been well coagulated. A hard-boiled, but not a raw, egg can be sliced for hors d'oeuvres. Cell masses also can be cut only after they have been similarly hardened, and usually further supported by impregnation with some molten solid, as paraffin or celloidin. The hardening of tissues is

achieved by allowing them to soak in protein coagulants; though for some purposes they are simply frozen solid and the cells cut while held rigidly in their own ice.

Another problem has to do with the optical properties of protoplasm. A solid object immersed in a fluid with the same index of refraction is perfectly invisible unless it be distinguished by color or some other unique character. Since solid inclusions in fluid protoplasm are mainly bits of gel lying in a sol of similar composition, refractive indices of different cell structures tend to be very similar. When examined under a microscope, therefore, a living cell or even one hardened and exposed by slicing may appear rather homogeneous. This difficulty has been circumvented by adding various chemical reagents which combine or react with specific substances composing the various cell structures. Particularly useful have been the dyes, which attach to characteristic parts of the cell and so make them clearly and sharply visible.

Cells, then, are ordinarily studied under the microscope not in the living state, but after having been violently mishandled. They are killed, chemically boiled, cut, and brilliantly painted before they are placed under the lens for examination. Now indeed much is to be seen, but now also arises the question whether these pictures represent even remotely the situation in the living cell. Scientific warfare has raged about this for many generations and the answer, as is usual in such cases, is equivocal. Some things are what they seem, others very much not.

In nearly all cells can be seen an oval or spherical region, usually somewhere near the center, which is sharply set off from the remainder. This has been called, from its position, the nucleus. In common tissue preparations, stained with the red acid dye, eosin, and the blue basic dye, hematoxylin, the nucleus is colored a deep blue, the cytoplasm of the remainder of the cell with a greater or lesser intensity of red. Now it is certainly not true that living cells have blue nuclei but it is no less true that nuclei are present, because this striking cell

structure can be seen clearly in most living uninjured cells. It must further be true that nucleus and cytoplasm are chemically different, else why should each stain with a different dye? It is even possible, though far from safe, to guess that the nucleus
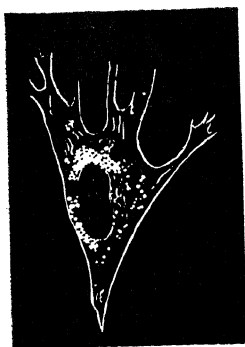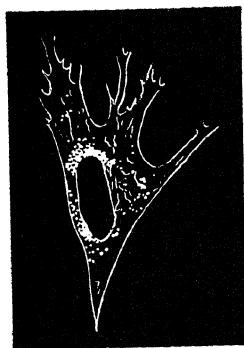
FIG. 121. At left, living fibroblast (top) seen under dark field illumination, compared with the same cell after killing with osmium fixative (below). (From Gray, after Strangeways and Canti.)

At right, living cells with chromosomes visible (top); the same cells after killing with osmium vapor (middle); and, finally, after staining with special dyes (bottom). (After Belar. From Darlington's *Recent Advances in Cytology,* T. & A. Church. II.)

contains an excess of acidic molecules, which combine with the basic dye, while the cytoplasm possesses more basic ones, which combine with the acid eosin.

The actual structures visible in treated cells are often not seen in living ones, and certainly during life rarely have the form seen in the dead carcass. But, no less certainly, some regional differences must have been present in the intact unit which developed into the visible structures on treatment. The situation is entirely analogous to that in an exposed but unprocessed photographic film; no visible differences exist between the different parts of the surface, yet, since the picture does appear on development, the pattern must have been present all the time. The difficulty arises when attempts are made to extrapolate back from the condition in the fixed cell to that in the living one. True, each fixed structure represents something, but just *what* is sometimes a difficult and occasionally an unanswerable question.

The way out of a theoretical dilemma is usually to obtain additional information, and when one experimental or observational approach has been fairly well exploited, progress results when a new one is devised. Several new methods of attack on the problem of cell structure have been introduced since the major technique already described was established. For one thing, the most serious changes in protoplasm are those associated with its death and coagulation. So long as cells are clearly still alive, the structures visible in them probably exist as seen. Some few dyes are able to penetrate the membranes of living cells and are sufficiently non-toxic so that life continues even when considerable amounts have entered the protoplasm. Such "vital" dyes, like those applied to fixed cells, serve to throw into optical relief the different structures in or on which they become especially concentrated. A paramecium so stained with neutral red, for example, continues to swim about actively, though its blushing interior exposes clearly defined nucleus, vacuoles, and other structures to the exploring gaze.

The visibility problem is solved in another way with light of

C
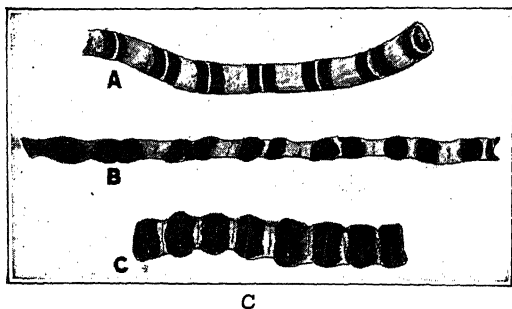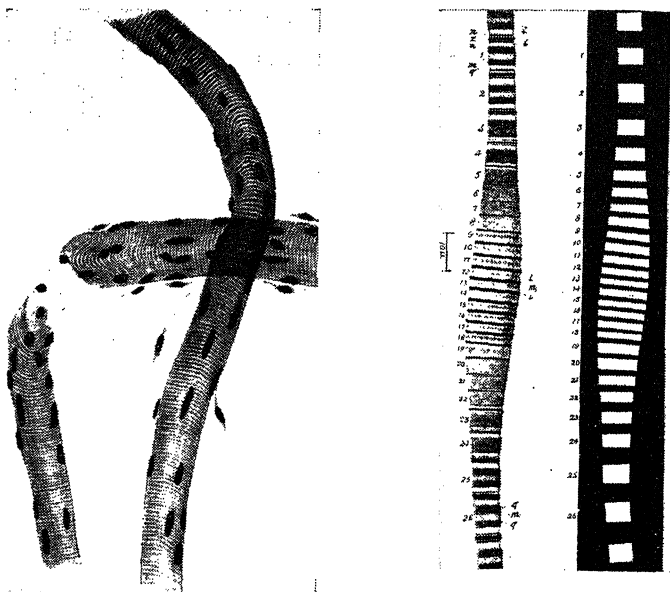
FIG. 122. Different preparations of striated muscle fibres. A, usual stained preparation of vertebrate fibres; B, vertebrate fibre in ordinary and in polarized light; a wave of contraction is shown. C, wasp fibres relaxed (top), stretched (middle), and contracted (below). D, relief photograph of two vertebrate fibres.

(A, from Maximow and Bloom's *Textbook of Histology*. Copyright W. B. Saunders Company. B, from Bayliss' *Principles of Physiology*, Longmans Green & Co., Ltd. C, after Schäfer, from Mitchell's *Textbook of General Physiology*, McGraw-Hill Book Company, Inc.)

D
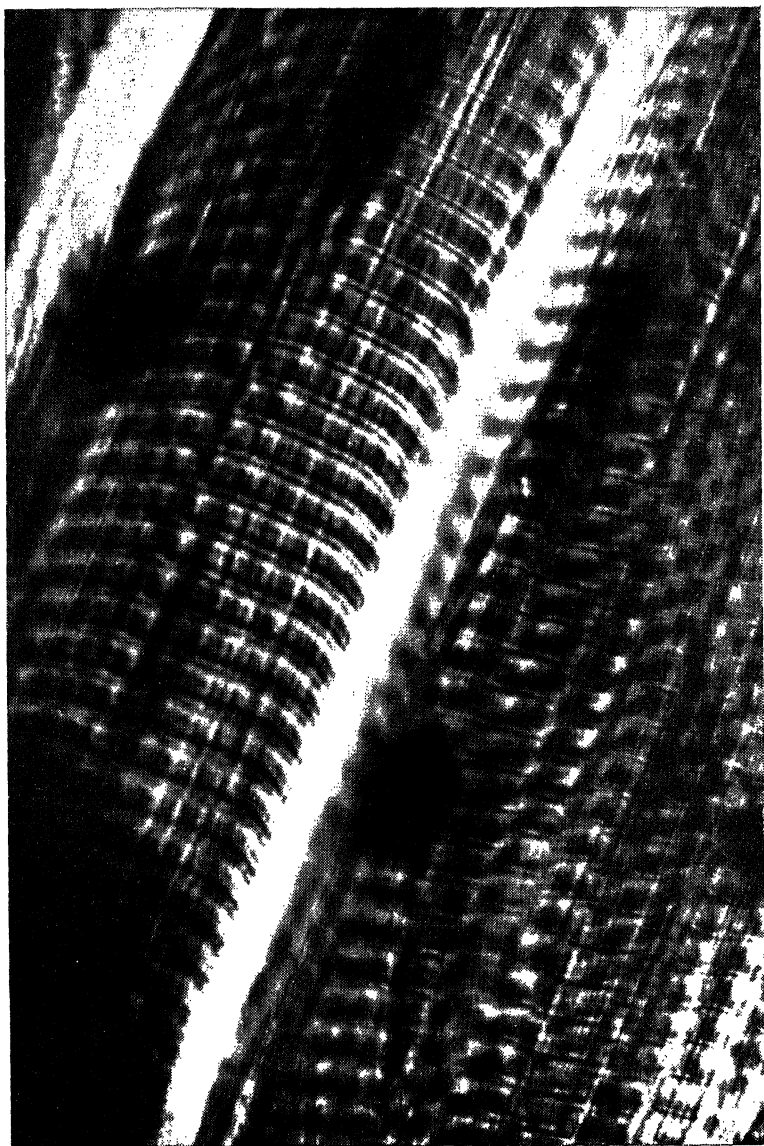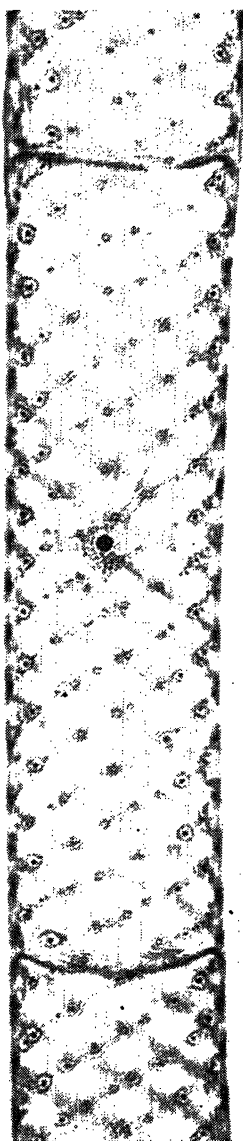
wave lengths different from those of ordinary light. Substances which are quite transparent to visible light may be opaque to one or another part of the ultraviolet spectrum. A band of wave lengths in the ultraviolet will, therefore, be absorbed differentially by different cell structures, which is the equivalent of being colored differently in ordinary light. True, the resulting picture is invisible to the eye, but it can be recorded on photographic plates and in other ways. Instead of ultraviolet light, polarized light in the visible range can be used in much the same way. In such light the direction of vibration of the light rays is uniform and the rays will, therefore, easily pass through, or be seriously interfered with by, a palisade of molecules also arranged uniformly. If the plane of vibration of light is favorably oriented with the plane of alignment of molecules, maximal transmission occurs; if at exact right angles to this, transmission is minimal. Since different cell structures are placed at varying angles relative to the light beam, those with oriented molecules transmit more or less of the rays and are delineated by grades of light and dark.

Still another modified illumination

FIG. 123. Microphotograph of a single alga cell (spirogyra) enlarged about 200 times.

is obtained with the fluorescence microscope. Many fluorescent substances are present in cells or can be introduced into them like dyes, often in the living state. Such a substance has the property of emitting visible light when invisible ultraviolet light falls upon it. Each is then seen as a shining object, like glowing phosphorus or a luminous watch dial. Under this microscope, each separate structure containing some fluorescent substance is seen as a bright object glowing warmly with its own particular colored light. Though the cells are rapidly killed by the ultraviolet illumination, it is possible to study them for a reasonable time in the living and probably uninjured condition.

Of course, such indirect means of rendering visible the internal organization of the cell are not applicable to those buried in a tissue mass; but even here, various tricks have eliminated the absolute necessity of hardening and sectioning. Single cells, such as protozoa or blood cells, can be studied without trouble. Cells on or near the surface of a solid tissue can be satisfactorily examined by reflected instead of transmitted light—the difference between looking at a picture on canvas or through a silhouette on glass—and transmitted light can be used if it is sufficiently intense to penetrate the whole tissue thickness. Better, the light actually can be introduced into the tissue below a desired thickness of cells, by utilizing the extremely high refractive index of quartz. A beam of light entering one end of a quartz rod will follow the curves and twists of the rod with relatively little emerging from its smooth surface. If the other end of the rod be drawn to a point, nearly all the light will leave here and the point thus becomes an extremely intense source of cold light. Such a quartz tip pushed into a tissue will brightly transilluminate cells over considerable thickness, and those between the tip and the microscope lens are readily studied.

Still other ways of circumventing the opacity of a solid tissue involve the mechanical breakdown of the mass. Instead of making regular clean sections throughout the hardened tissue, the fresh material is placed under a low-power microscope,

with a binocular arrangement to permit the estimation of depth, and with fine glass needles as instruments, individual cells or small groups can often be teased apart with very little injury. Or, by tissue culture, perfectly healthy growing cells can be obtained in relative isolation. Bits of the desired tissue are placed, with aseptic precautions, in a drop of fluid containing the appropriate salts and nutrient substances, usually on some sort of gel for mechanical attachment. The whole is kept warm and supplied with oxygen and the cells survive and flourish. As they grow, they gradually spread along the surface and eventually form a layer one or a few cells thick. This method permits the study of cells in many ways, including the various optical ones.

There remain yet other ways of skinning the cat. It has already been mentioned that tissues can be hardened by suddenly freezing them, and sections made of the frozen block. Even freezing, however, directly disrupts structure or, more important, produces such ultramicroscopic damage that the cells are killed and undergo considerable change when they thaw out. But if water could be completely removed from the cell, the dry mass left could be handled in the usual way. Simple desiccation is useless because, as water evaporates, the remaining material shrinks together and becomes entirely distorted. A combination of freezing and drying avoids these troubles.

Moderately thin tissue bits plunged into liquid air or other very cold liquids are frozen so rapidly that almost no change occurs other than the conversion of the cell water into ice. The frozen chunks, kept well below the freezing point of water, say —20°C., are then kept in a vacuum until they are completely dry. Fortunately, ice can sublime directly into water vapor without passing through the liquid state; as shown by the gradual disappearance of snow and ice from the ground during clear persistently subfreezing weather. Cells so treated are close to normal as regards all constituents except the water, which is gone. The original structures and configurations are

well retained, the whole can be sectioned or coarsely powdered and directly studied under the microscope. Further, so long as no water is added, all sorts of additional chemicals can be applied and a fairly accurate picture obtained of the initial composition of the cell. Specific substances can be accurately located with the aid of appropriate reagents that test for them; starch granules, for example, will turn blue if iodine vapor is allowed to act on the dry mass.

A somewhat related procedure for studying the position in cells of particular substances is microincineration. In this case, the frozen and sometimes desiccated tissue is sliced in the usual manner, a section placed on a glass slide, and the whole rapidly heated. This treatment not only drives off any water but burns away all the organic matter as well. Only the salts are left behind as delicate ash which more or less perfectly silhouettes the original cell mass. This salty wraith is not homogeneous but retains the pattern of many of the cell structures. The ordinary cylindrical cell of voluntary muscle exhibits a banded or striated appearance even in the living state, and this is very striking in the usual fixed and stained preparation. The cylinder seems to be built up of similar discs piled upon each other. After incineration of such a muscle cell the same striations remain traced in fine lines of crystals chemically identified as potassium salts. In so far as the method is reliable, and there is some doubt of this, it follows that the potassium in muscle cells tends to be concentrated at a certain plane in each of the transverse discs.

FIG. 124. A muscle fiber has been incinerated on a glass slide and the ash tested for potassium salts with this result. The striations, membrane and nucleus seem to contain all of this substance (After Scott.)

These same muscle cells show a longitudinal striation after fixation, as if the whole cylinder were a sheaf of fine fibres, each built up of the transverse discs. There has been much debate
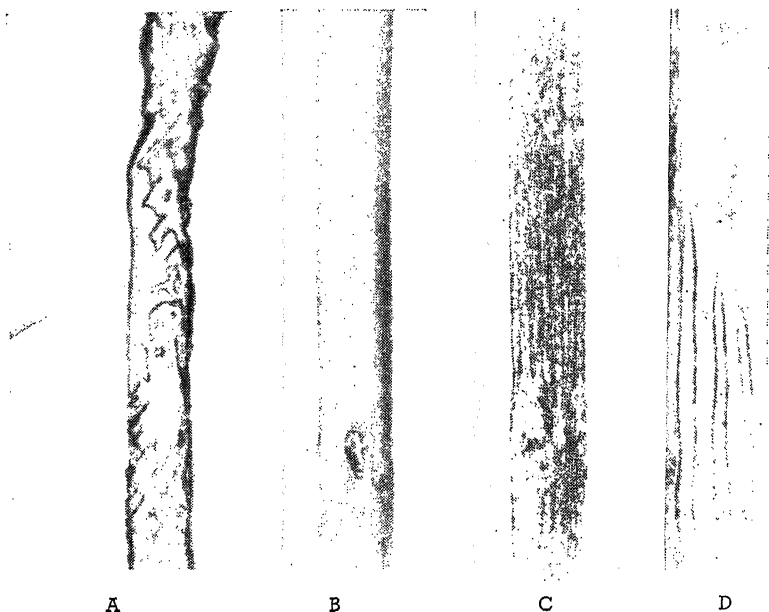
FIG. 125. Microphotographs of micromanipulation. A, single living striated muscle fiber with plates of ice on its surface but the protoplasm not frozen. Microneedle ready to inoculate ice. B, living fiber kept at —3°C. for 45 minutes. Not yet pierced, no ice. C, same fiber as B at —2°C. some minutes later. Inoculated with ice needle just before. D, another fiber at —5°C. Three ice columns are forming up its length following a puncture below the edge of the picture. (Photographs by courtesy of Robert Chambers.)

as to the real existence of such myofibrils, and again a modified freezing method has helped to solve the problem. If such a muscle cell is placed in a drop of neutral oil on a microscope slide and the whole cooled to somewhere between —5°C. and —10°C. nothing happens. Ice crystals do not form even though the water is supercooled. When a supercooled liquid is inoculated with even the tiniest crystal of its solid form, however, the whole mass almost immediately solidifies, and this is true for the muscle water. If the cold cell, therefore, is suddenly punctured with a minute icicle, used as a dagger,

its water turns to ice almost at once, and the growth of crystals can be watched under the microscope. The crystals do not radiate evenly in all directions from the point of puncture, but rather form in parallel sheets or rods which run the length of the cylinder. Clearly the water is not homogeneously distributed throughout the cytoplasm of the muscle cell, but is confined to longitudinally oriented spaces such as would exist between longitudinal fibrils of denser protoplasm.

Operations on Cells

It is simple to think or talk about stabbing a single cell at some desired point with a miniature ice dagger, but quite another matter to translate this into action. That this is possible is due to a refinement of another entirely different method for studying cell structures, that of micromanipulation. A cell is handled or manipulated with the aid of tiny instruments, the whole procedure being watched and directed through the microscope. The problems are, of course: first, to obtain appropriate instruments of sufficiently miniature working dimensions; and second, to control minutely their movements near or in a stationary cell.

For many purposes, the uninjured cell can be held quite rigidly in the vice of surface tension. With a sufficiently small amount of liquid about it, the fluid surface presses the cell against the glass slide and firmly anchors it. Care must, of course, be taken to prevent evaporation, first of the surrounding fluid and then of the cell water, by keeping the whole in a moist atmosphere maintained in a semi-closed chamber. The instruments can then be moved about through the air or liquid until they reach or penetrate the cell. Whatever the form of the working termination, these microinstruments are composed essentially of conveniently sized glass tubes or rods with one end drawn out and so narrowed to microscopical dimensions. The shank of the rod is held in a clamp attached, through a

series of movable parts, to the microscope itself. The holder is so arranged that separate adjustments in each of the three planes can be made by hand screws or other mechanical means. The needle tip is thus swung about through space along three coordinates; and with a little practice it is possible to direct its motion under the microscope smoothly and precisely. Two such complete units are ordinarily used, so that each hand maneuvers its own needle.

Solid needles are easiest to prepare and, with their minute dimensions, they cut much like knives. With them it is possible to tear open the cell, much as one might a plum, and allow the intact nucleus to float out from the debris. When the nucleus is in mitosis and the chromosomes are present, single ones can be dissected out and their existence as discrete entities so demonstrated. Even when the chromatin is in the form of a fine tangled thread, it has proved possible to catch a loop of this on a needle and stretch it out like a delicate elastic. The thread, which had been seen in cells only under certain special conditions of preparation, is thus proven to be a real physical structure and not an artifact resulting from the treatment used to make it visible.
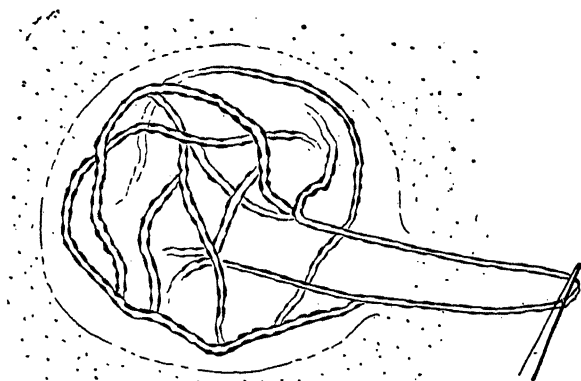
FIG. 126. With a microscopic needle it is possible to pick out and stretch a thread of chromatin in the nucleus of a living cell. (After Chambers.)

A needle pressed against the surface of a red blood corpuscle pushes in this surface, as a finger indents a hollow rubber ball; and, like the finger and ball, when the needle is removed the cell resumes its original shape and the needle comes away clean. It, however, the cell surface is punctured or cut it is no longer possible to remove the needle so easily. As it is withdrawn the cell tends to drag after it, for the glass has become well stuck with viscous cell contents. The protoplasm can be drawn out into long strands before they snap, leaving a small gummy mass on the needle. Clearly the red blood cell has some sort of surface membrane, a definite structural entity with properties different from those of the contained cytoplasm. When the same experiment is repeated with a white blood cell the results are different. Now the needle need merely be pressed onto the surface to become stickily attached. So far as this test is concerned, then, the white cell lacks the special non-sticky membrane possessed by its red cousin.

Much other impressive information about cells has been garnered with these microneedles, which can only be mentioned here. Two needles stuck into opposite ends of a single muscle cell are pulled toward each other when this contracts, and the strength of pull and distance of movement have been so measured. An amoeba can easily be cut into two parts, each of which rounds up and continues to move about for some time. The portion containing the nucleus soon is rebuilt into a perfectly intact animal which grows and reproduces; the other, though it may live for a day or two, inevitably dies off, apparently starving to death after consuming its own protoplasm. Here is evidence for the fact, stated earlier in a categorical fashion in connection with the degeneration of cut nerve fibres, that any part of a cell separated from the nucleus is irrevocably doomed to death.

The tremendous importance of various salt ions in controlling the colloidal state of protoplasm has also been dramatically demonstrated by microdissection technique. If an amoeba, sus-

pended in a salt solution containing the proper balance of
potassium and calcium ions, is slashed into by a needle the
damage is slight. As described earlier, cytoplasm starts to pour
out through the wound but gels as it meets the salt solution, so
that the process is soon stopped and the normal cell membrane
promptly reformed. A similar cut in a solution with excessive
potassium prevents the cytoplasm from gelling and the amoeba
disintegrates. When the nucleus emerges it remains intact
unless punctured, in which case it suffers the same explosive
disintegration—evidence for the existence of a definite nuclear
membrane. With excess calcium, the protoplasm coagulates
instead of gelling into a semipermeable membrane, so that cal-
cium ions diffuse through it into the body of the cell, coagu-
lating and shriveling as they go. What a responsibility for
the few layers of molecules that clothe the protoplasmic interior
of a living cell! While this barrier is intact, considerable differ-
ences in external salt concentration are innocuous; when it is
ruptured, the protoplasm explodes or collapses, depending on
the way the ions are stacked against it.

The microsurgeon's armamentarium is not exhausted with
these needles and scalpels. Even more useful are the micro-
pipettes or capillaries obtained when, instead of a glass rod, a
glass tube is drawn out to fine dimensions. Such a capillary,
filled with water and then cooled below freezing, develops at
its tip the tiny icicle whose use we have already seen. More
commonly, the fluid-filled capillary is quickly jabbed into a
cell and used either to withdraw a portion of the cell contents,
as the limpid fluid of vacuoles, or to inject desired reagents.
These chemicals may react with particular substances in the
cell, which are thus identified as already discussed. Most often
dyes have been injected, often separately into the nucleus and
the cytoplasm or some other structure, and the cell chemistry
deduced from color changes.

Many dyes shift from one color to another when some criti-
cal degree of acidity or alkalinity is reached. By injecting a
sufficiently wide array of these, the hydrogen ion concentra-

tion, or acidity, of different cell regions can be estimated fairly well. Other dyes change color as they are oxidized or reduced and so give fairly definite information concerning the oxidizing or reducing level of particular organelles. Or, by injecting a dye into one region of the cell and watching its spread, evidence can be obtained as to the existence of impermeable membranes or other barriers. Gas or oil droplets have also been introduced into cells and their movement through the structures, especially under centrifugal force, has been followed. Incidentally, microchemical methods of such delicacy have been devised that several substances in the sap drawn from within a cell, or the extracellular fluid obtained from beside it, can be measured quantitatively.

Capillaries whose channels have been filled with salt solution or with delicate metal wires conduct electricity and serve as microelectrodes. They have been used both to apply currents to cells and to measure potentials and currents generated by them. The large algae cells especially lend themselves to such treatment—one electrode has been jabbed through the surface, another one left outside, and the polarization of the surface membrane directly measured by the voltage difference they register. Further, the effects of stimulation and of injury on this polarization potential have been observed; indeed the results of such studies offer the most direct evidence of the existence and behavior of charged cell membranes on which theories of excitation and conduction are based. Currents applied by microelectrodes can be graded as accurately as any others, and the laws of stimulation have so been shown to apply to single cells. It has even proved possible, using a double pipette with a loop of resistance wire across the end, to perform microcautery operations and burn out definite parts of a cell. A like arrangement, but with two dissimilar metal wires in the capillaries, constitutes a microthermocouple with which temperature measurements within a cell have been made. And so with other physical attributes of cells, themselves rather directly dependent upon structural organization—resistance,
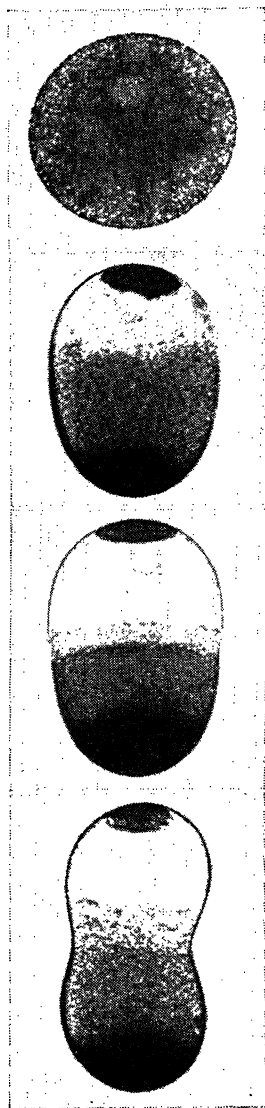
capacity, inductance, conductivity, viscosity, and so forth—means for measuring have been devised.

Cells have been handled mechanically without the use of micromanipulation apparatus. A single egg can be squeezed between two plates with a known force, the resulting flattening measured, and the surface tension and elastic properties of its membrane calculated. Centrifugal force can be applied to the insides of a cell while the whole remains stationary. Eggs suspended in a fluid of exactly their specific gravity do not move up or down when centrifuged. But the heavier constituents within each cell are thrown to its distal surface, the lighter ones to its proximal one. A microscope built into the arm of a centrifuge has made it possible to watch the changes within cells even as they whirl dizzily many hundred times a second. An ordinary egg soon stratifies into three layers: a light oil drop, which moves toward the center of rotation and comes to rest against the proximal membrane; heavy yolk granules which accumulate opposite them; and an almost

FIG. 127. Microphotographs of a living sea-urchin egg in the centrifuge microscope (magnification about 400 times). A, normal; B, C, D, progressive changes with centrifuging. (Photographs by courtesy of Ethel Browne Harvey.)
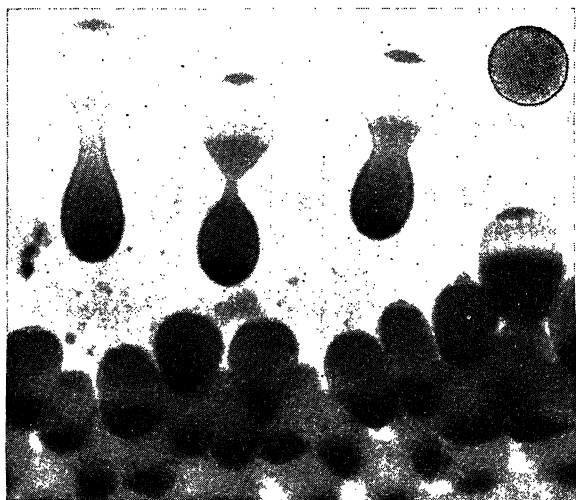
FIG. 128. Microphotograph of several urchin eggs about to separate into halves while being centrifuged. Below are the heavy halves, of eggs already separated, which are now becoming stratified preparatory to forming quarters. A normal egg is shown at the upper right at the same magnification (150 times). (Photograph by courtesy of Ethel Browne Harvey.)

structureless layer of cytoplasm remaining in the center. The nucleus is light, for it moves up with the oil. As the cells are spun faster and faster, the differential centrifugal pull on the light and heavy ends becomes sufficient to stretch the intervening membrane and elongate the whole egg; and with further acceleration the stretch becomes great enough so that each cell, like any drop of fluid sufficiently stretched, finally pinches in two near the center. Two half-eggs are thus formed from each normal one and, with very high speeds indeed, each of these can similarly be made to divide into quarter ones.

The materials originally scattered throughout a single cell thus become segregated, solely on the basis of their difference in specific gravity, into two or four categories. Rather amazingly, each of these fragments of an unfertilized egg can be fertilized by a spermatozoon and begins a normal development. Those without a nucleus do not continue very far, to be

sure, but it was a surprising discovery that an enucleated egg can divide even a few times. The metabolic properties of the centrifuged fragments have been determined and, for example, the nucleated half shown to have a higher rate of oxygen consumption than does the heavier one. Other kinds of eggs, centrifuged less rapidly so that they remained single units, have been fertilized and allowed to develop. The usual rapid cell divisions occurred and an embryo formed with the proper cell types present. The cells were, however, woefully out of place; those destined to form the skin, for example, lay in a clump in the center instead of neatly spread around the outside. Important conclusions about cell differentiation during development follow from these experiments and must soon be considered.

Many other ways of studying cells could still be discussed— the measurement of permeability, the application of drugs, of high frequency vibrations (which explode them), of X-rays, of all sorts of electrical currents, and so on in an ever-increasing list—but it is now time, perhaps, to turn from the methods of study to a more explicit examination of some things learned by their use.
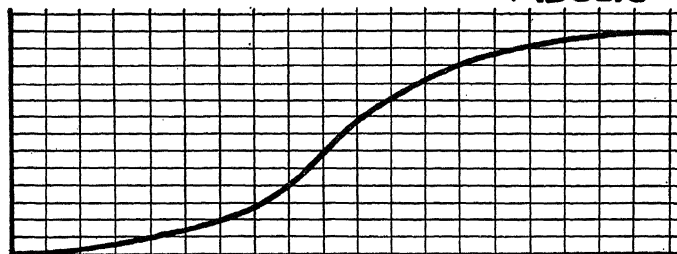
Chapter Ten: Reproduction Among Molecules—
Specific Synthesis

Contemplate two tiny globules of protoplasm resting on a rocky ledge below the ebb-tide mark. They look alike: two spheres not one-tenth of a millimeter in diameter, barely visible without a microscope and with it seen as fairly transparent, granular cytoplasmic blobs containing the nucleus and other structures that label them as cells. Not only do they look alike but if analyzed chemically they would be found to contain practically identical amounts of the same things: much water, salts, a little protein, and all the other simple and elaborate molecules that collectively constitute protoplasm. They are bathed in the same sea water, chemically much like the salt solution of which they are overwhelmingly composed. They are at the same temperature, receive the same sunlight, have washed about them the same food material; in fact their environment is as nearly identical as can well be imagined. Each is an egg cell, and when fertilized or set into activity by other means will proceed to develop in beautifully regular

fashion into an adult animal. Each cell will divide, grow, and divide in monotonous repetition while steadily increasing the total mass of protoplasm and molding it into characteristic forms. But the two animals that eventuate will be almost ludicrously unlike, for one cell is the egg of a starfish, the other that of a sea cucumber.

Clearly, the eggs were different from the start, since the environment was alike for both, and each must have possessed some property unique to its kind. If our microscopic examination and chemical tests fail to reveal this we must find a more delicate means of investigation or hide our ignorance behind such sterile phrases as "different vital forces," "special guiding principles," or "individual directing entelechies." The adult animals have like cells, in different arrangements to be sure, and even cells that appear to be strikingly different in kind; yet they are all, like the original eggs, built of familiar protoplasm with its familiar composition. Are these cells truly different from one another or are they merely different states or phases of like cells? The same umbrella looks very different open or shut. If the cells are indeed different in kind, with enduring individuality, then surely they must be composed of protoplasms which are also individual and which possess certain unique chemical ingredients. But then no less certainly must the original eggs have possessed a separate chemical identity, for each, under similar conditions, formed a different adult constellation and each was, in fact, produced by a different adult ancestor.

Each species or race of living things reproduces its kind with the utmost fidelity from one generation to another. Each adult possesses a wide array of cells or cell parts, and it seems a reasonable hypothesis that each of these is chemically specific in some way. Further, the specific chemicals of large masses of cells must have been formed somehow under the influence of those in the original unit. In other words, specific substances have been synthesized. Our problem, then, is to find ways of

identifying these individually characteristic materials and, if possible, to solve the trick of their specific manufacture. If the microscope and test tube cannot yet discern the differences in these substances, it would seem a reasonable hunch to try to make the organism itself perform the selection, for cells obviously are "aware" of differences in protoplasms. This will lead us into a long but fascinating detour before returning to the paramount question of how like creates like.

Chemical Defense

Diseases of one large group tend to afflict man in childhood but, having once struck, will not normally recur in the same individual. An attack of diphtheria confers immunity to any further diphtheritic invasion. Measles, whooping cough, or chicken pox, each locks a door behind it so that it cannot reenter; yet the child immune to chicken pox will acquire measles with no more ado than any other tot. Nor are such phenomena limited to the diseases of youth; they are manifest in a large variety of other infectious diseases. Let us take typhoid fever again as an example, since we have already seen something of the typhoid bacilli. Most people never catch typhoid fever, not, to any great extent, because they have a superior resistance to the disease, but rather because they do not come in contact with it. Typhoid has many times viciously swept over a continent, attacked a high proportion of the population, and left large numbers of the victims dead.

The typhoid bacilli pass from an infected individual to a healthy person by direct contact, on handled objects, via sewage, and particularly in water and milk. They live for a considerable time in polluted water and thrive when accidentally inoculated into commercial milk supplies. Healthy individuals swallow the living organisms in food and drink, the bacilli find just the right biological climate in the intestines where they are so conveniently deposited, they grow prodigiously and, so

to speak, bite the gut that feeds them. A new case of typhoid fever soon develops and the bacteria eventually get into the blood and so all through the body. The host for these parasites may succumb to an overwhelming invasion and die. More often, fortunately, the body successfully fights off the invaders, destroys them, and recovers. Not infrequently, though the typhoid germs within the tissues of the body are successfully destroyed, some manage to survive for long periods in protected nooks and cavities, such as the gall bladder, from which small numbers of living organisms may continue to find their way into the outer world for many years.

The persons harboring such surviving colonies, though themselves quite healthy, continue to carry and spread the disease; and medical and public health records abound with fantastic stories of how the movements of such a carrier from place to place about the country have been recorded in one outburst after another of local typhoid epidemics. Whenever the stringent public health measures of our modern urban civilization break down, typhoid breaks out; and, indeed, the efficiency of the sanitary direction of a city is about as well measured by the typhoid rate in the region under its jurisdiction as by any other indices. Interference with the continuous rigid control of sewage, drinking water, and the like, as occurs, for example, following disastrous floods or fires, brings a typhoid epidemic in its wake. Congestion of large numbers of people without adequate sanitary engineering, as regularly occurred in the armies of the past and even does sometimes in those of the present, can be depended upon to bring about a typhoid epidemic.

Clearly, then, the great majority of people will contract the disease if given an opportunity. Yet those who have had and recovered from typhoid fever can safely work among typhoid patients, can eat contaminated foods, can even deliberately swallow the bacteria, and remain untouched. For typhoid fever confers immunity upon those whom it attacks yet spares.

Something has obviously changed in an individual who had been susceptible, suffered the disease, and has been thereafter and is henceforth immune to typhoid. Similar, but not identical, changes must have occurred in those other patients who have become immune to each one, or any combination, of the other immunizing infections.

There are many conceivable ways in which this immunity could be brought about, and an enormous amount of study has gone into its clarification. We are especially interested here in one of these possible mechanisms. Might the immune person produce some substance which is inimical to the attacking organism? If so, we should expect a person recovered from typhoid fever to possess an anti-typhoid substance; one over an attack of scarlet fever, an anti-scarlet fever substance, and so on. If each infecting organism caused the body to generate its own particular anti-substance or antibody, the facts of immunity would be accounted for.

Note, though, the remarkable conditions that must be met. The human or animal body, for immunity occurs entirely comparably in other animals, must first somehow identify the invading organism and then proceed to manufacture a particular antibody specific for and potent against this organism. Of course, a typhoid bacillus, a diphtheria bacillus, and a scarlet fever streptococcus are quite different cells and can easily be distinguished under the microscope and by many other laboratory methods. But the invaded organism distinguishes just as surely among many races of streptococci which cannot be discriminated in the laboratory, as it does among these more familiar invaders. Here, then, is a possible way in which organisms may be made, themselves, a test for differences in other organisms.

Looking backward from our present vantage point, we see that the simplest sort of experiment can demonstrate the presence of antibodies in immune animals. If blood is drawn from a normal person and allowed to clot, there remains a clear
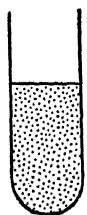
NEGATIVE

POSITIVE

Fig. 129. Widal test for typhoid antibodies by adding typhoid bacilli to blood serum.

faintly yellow liquid called serum. This normal serum has no effect on typhoid bacilli when mixed with them in a test tube or on a microscope slide; the bacteria continue to swim around in it as separate active individuals and remain quite healthy. The suspension of bacilli in serum or salt solution looks slightly cloudy and remains so for hours or days. If the same experiment is repeated with serum from the blood of a person immune to typhoid fever, following an attack of the disease, the results are very different. The general cloudiness of the bacterial suspension is lost almost at once and little solid flecks appear throughout the liquid and then rapidly settle to the bottom. Under the microscope these flecks are seen to be composed of large numbers of individual bacilli which have become stuck or agglutinated together. Some antibody which agglutinates typhoid bacilli is present in the immune serum but not in the normal. Entirely similar agglutinins appear in sera from persons immune to diphtheria or any of the other diseases and, as postulated, each of these antibodies reacts only with the particular organism that caused the previous infection. An immune serum containing anti-typhoid agglutinins behaves toward diphtheria bacilli as does any normal serum, and vice versa.

We look again for the clump of typhoid bacilli thrown down by the agglutinin and may be surprised to find it has entirely vanished. The bacilli which were first clumped are now dissolved and, had we continued our watch through the microscope, one after another of the microbes would have been seen apparently to explode. Either the same or another antibody in immune serum confers upon it this property of breaking up or lysing the bacteria and is hence called a bacteriolysin. Still

other and more surprising actions appear in immune sera but, in essence, the ones we have observed are characteristic of the lot. The point is that certain definite chemical substances, which appear in the blood of an animal following the entry of some one kind of bacteria into that blood, have the ability to act upon and destroy just that particular organism.

Biological Reagents

But definite substances are chemical entities with certain fixed characteristics, such as solubility and the like, so that it should be possible to further separate and purify the antibodies of immune serum. This has been done. And in every case the antibodies have proved to be proteins, indeed, proteins belonging within one particular subclass of these substances, the so-called globulins. It is hardly surprising that these antibodies are proteins for they show two of the most important characteristics of enzymes and may well be looked upon as a special category of these protein substances. The antibodies are highly specific and minute amounts of them bring about rapid and extensive chemical changes.

Immune sera containing extremely powerful antibodies often show no measurable change in the amount of their globulin, initially only 2 or 3 per cent of their mass, so the amount of antibody present in a drop of such serum is manifestly extremely small. Yet such a serum may be diluted ten million times and still retain its specific action on the organism to which it is immune. Further, the changes produced by these antibodies are chemical in the last analysis, for lysis not merely involves the destruction of the cell membrane and the scattering into solution of various molecules found in protoplasm but, still more, it includes a direct splitting or hydrolysis of these molecules, as of proteins to peptides and amino acids. (The initial agglutination is a physico-chemical effect due largely to

a change in the charges on the surface of the bacteria and is entirely comparable to the precipitation of colloids by coagulating agents.)

The antibodies, then, are a sort of enzyme formed by the body in response to infection. They would hardly be produced in the blood plasma, which is no more than a complex solution of chemicals and has none of the properties of "living" protoplasm; nor yet in the red blood corpuscles, which are hardly more than sacks of hemoglobin. No, some fancy molecular embroidery is needed and this must be done by living cells. The problems then arise: how are the appropriate cells stimulated to this creative effort and, of lesser consequence, how are the large protein molecules built within them passed through their impermeable membranes and dumped into the circulating blood? This second problem must be passed by; it is one aspect of the more general one of the secretion by gland cells of special substances with large molecules.

Considering the first question, the antibody-producing cells must be stimulated by something actually reaching them. This might be the bacteria themselves or some substances given off by them or, perhaps, nerve messages or chemicals released by the action of the bacteria elsewhere in the body. An indirect stimulus is easily disposed of as a possibility, for when bacteria are kept localized in certain tissues, the antibodies are found at first only in that locality. The question resolves itself, therefore, into two fairly tangible ones: what substance reaches the cells and stimulates them, and how does it do so? The first of these, of course, involves the problem of specificity, for the antibody to be formed is unique and appropriate to each kind of stimulator.

It would be a pretty shrewd first guess to look again at the familiar proteins; its proteins more than anything else distinguish the typhoid bacillus from all other cells. The experiment is simple enough in principle. Fragment typhoid bacilli in some way, extract their proteins in a more or less pure form, and try

the typhoid protein solution, rather than the original typhoid bacilli. Such extracts injected into animals cause the typical typhoid antibodies to be generated. The test is, in fact, performed on humans, for injection of the killed bacteria leads to an immunity to typhoid fever without the danger and distress of a full-blown attack of the disease. So successful has this proved that millions of people are now being protected against typhoid fever by injection of a vaccine of killed and otherwise prepared typhoid bacilli. The same answer has come clearly and consistently from all sorts of tests on all sorts of animals: the true generator of antibodies, the antigen, is a protein, or at most a compound of protein with lipin or carbohydrate.

This discovery at once opens the door to a far wider application of these immune reactions, for if the proteins from typhoid or scarlet fever or other bacteria can serve as antigens and stimulate body cells to produce specific antibodies, why not a hoard of other proteins likewise? Again the scientific hypothesis is supported by experiment. Practically any protein, other than those normally present, when introduced into the blood of a living animal leads to the appearance in that blood after some days of antibodies which precipitate and eventually dissolve it. Here, then, made to order, is the very biological test we need. If a rabbit is first injected with one particular protein and later its blood is tested for the ability to precipitate a variety of proteins, the serum will react only with that one which served to "immunize" it. The reaction is highly, but not absolutely, specific; other proteins, chemically very similar to the original antigen, may be attacked to a slight degree. This leads to no confusion but rather still further enhances the usefulness of the test.

A rabbit is immunized, for example, with human blood serum (containing its specific proteins). The immune rabbit serum, when subsequently tested, even after great dilution, gives a clear precipitate of the proteins of any other human serum. It will not alter the serum of a dog, a cow, a horse, or what you
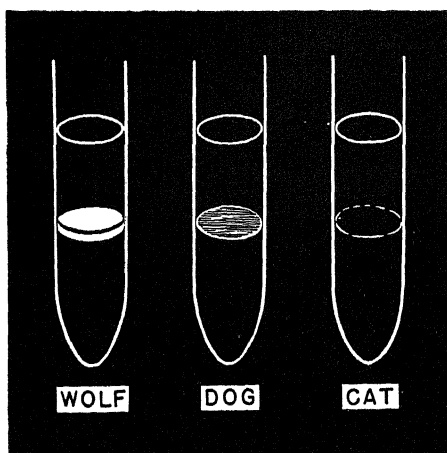
Fɪɢ. 130. Rabbit serum containing antibodies for wolf blood gives a heavy ring of precipitated protein when in contact with wolf serum. It does not react with cat serum but does so slightly with dog serum, which contains proteins chemically similar to those of the wolf.

will. Such immune serum can, therefore, be used as a specific reagent to demonstrate human blood proteins—and is the basis for the medico-legal examination of a suspected stain to prove whether or not it is made by human blood. Since the specificity is not, however, perfect, perhaps less diluted immune serum would react with other proteins very similar to those of the human. The blood proteins of the dog, cow, and horse, though indistinguishable from the human by the usual laboratory tests, are not sufficiently close to it to react even with concentrated rabbit's serum.

Is it surprising, however, to find that the blood of monkeys and still more that of apes will show some reaction with rabbit's serum immunized only to the blood of man? If the great structural similarity between these animals means anything it surely implies that their proteins are more nearly alike than are those of structurally less similar organisms. In the same way, serum immunized to dog blood will react moderately with that of the fox and faintly with that of the wolf. Clearly these immune
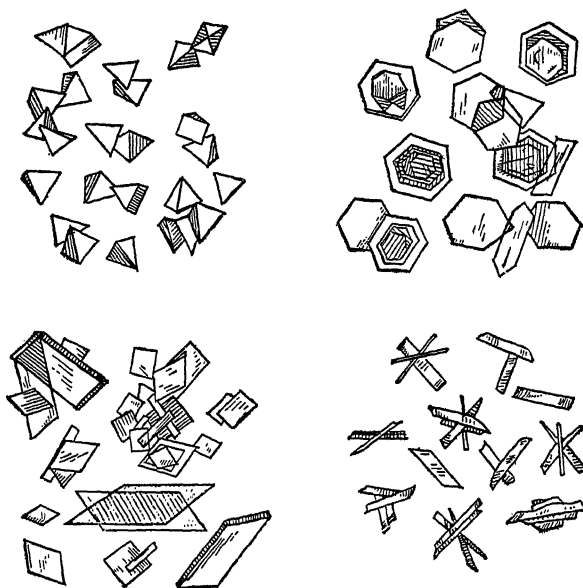
Fig. 131. Several types of hemoglobin crystals from the bloods of various species of animals. (After Reickert. From Mitchell's *Textbook of General Physiology*, McGraw-Hill Book Company, Inc.)

tests give an entirely new validity and precision to the concepts of "blood relationship" and inform us, in the most direct and objective manner possible, that man is indeed a blood cousin to the ape, just as the dog is to the wolf. Presumably this blood relationship, like any other, is the consequence of a common ancestry, and the more recent the common ancestor the more alike are the blood proteins. But just this is the main theme of the theory of organic evolution, now powerfully supported from an entirely new and unexpected source.

By such tests, then, the proteins of various cells of different organisms can be identified and compared. It turns out that there are proteins unique not only to every species of plant and animal but, even within one animal, to its various kinds or races of cells. The proteins of human liver cells differ from those in the human brain or kidney, just as they do from those

in the liver of an elephant. And the question of specific synthesis with which this chapter started becomes largely a question of the origin of these proteins in their appropriate cells. But there is further insight to be had from these immune reactions.

Bricks from Straw

The second question, how do the antigenic proteins stimulate cells to form the appropriate antibodies, remains to be answered. And this requires another, though briefer, detour into a related realm of biology. Let us return to the typhoid bacilli and follow their fate in the body rather than in the test tube. A suspension containing many billion bacteria injected directly into the blood stream in the vein of an animal should soon be evenly distributed throughout the blood. A given volume of blood taken from any part of the body would then contain the same number of bacteria. This can readily be tested and the bacteria counted by mixing the blood into a nutrient pabulum, previously sterilized, and allowing each individual to grow into an easily visible colony.

Actually, the bacterial count per cubic centimeter of blood does not remain high but falls precipitously during minutes after the bacteria are introduced. The organisms are either destroyed as they circulate or are taken from the blood as it strains through certain organs. Again direct counts give the answer. The blood leaving the liver and the spleen, and to a lesser extent the bones, contains fewer bacteria than the blood entering these organs, whereas in other parts of the body this is not the case. Conversely, a given mass of liver or spleen, when removed, mashed, and tested by the colony method, shows a rapid increase in its content of bacteria which have been taken from the blood.

Under the microscope the whole melodrama is made clear. In the liver and spleen, particularly, the large and small arteries
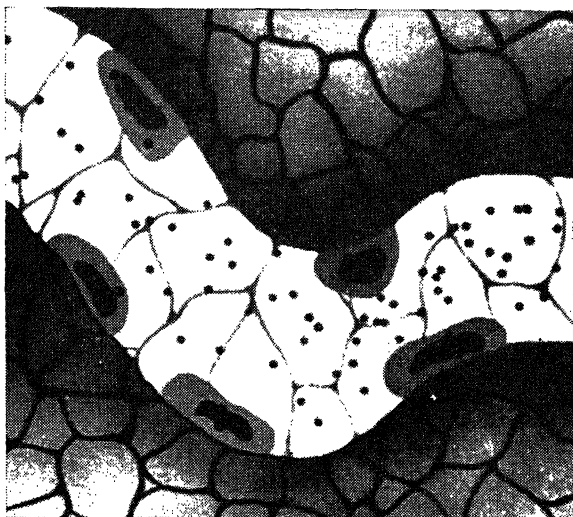
FIG. 132. Diagram of a single channel through a lymph node. The bacteria, floating slowly along, are taken up by cells lining the walls of each channel. (From the film, *Body Defenses Against Disease*, by Cannon.)

divide into capillary channels which are different from those through most of the body. Instead of fine cylindrical tubes, lined by smooth, flat and essentially inactive cells and through which the blood flows with considerable rapidity, the liver capillaries are enlarged, irregular sinuses, lined by thicker active cells, through which the blood slowly seeps. The circulation here is like that of a swamp rather than of a brook. The slow movement allows the suspended particles in the plasma—blood corpuscles, bacteria, particles of carbon if lampblack is injected, even single colloidal protein micelles—to bump irregularly and repeatedly against the cells lining the passage. And these active cells behave like fixed amoebae capturing food. When a particle hits and sticks to the surface, out come the familiar pseudopods to surround it and soon it is completely buried in the protoplasm of its captor. This process of phago-
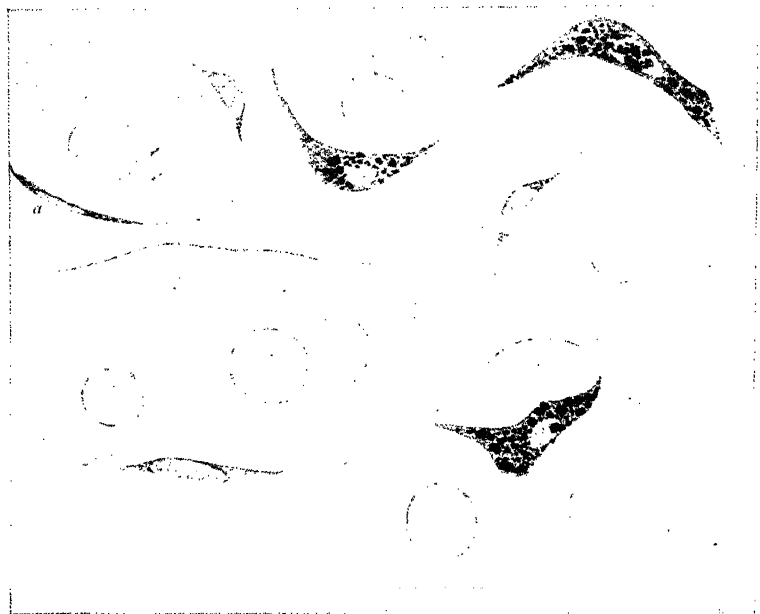
FIG. 133. Reticulo-endothelial cells lining capillary sinuses in the liver. Carbon particles (India ink) injected into the blood have been taken up by these phagocytic cells. (After Kupfer. From Downey's *Handbook of Hematology*, Paul B. Hoeber, Inc.)

cytosis, or cell-eating, applies to injured red corpuscles, and to all sorts of foreign cells or particles.

After the particle is phagocytized or ingested it is, if possible, digested. The microscope shows the same gradual loss of out-line and dissolution of the particle that it showed us earlier after the amoeba had taken its meal. These phagocytic cells, the ones that take up bacteria or foreign proteins and destroy and digest them, are, reasonably enough, the cells which produce the antibodies. In immunized animals these cells take up and digest the antigen far more expeditiously than they do in normals. Now note; to digest particular proteins the phago-cytic cells must possess the appropriate specific enzymes. As a result of having digested some protein, of being immunized,

the cells come to contain more of the specific digestive enzyme for this protein, as witness their more powerful action upon it. In fact, a considerable production of such enzymes must have occurred, because not only do more of them remain in the cell, but just such protein-digesting enzymes, or bacteriolytic antibodies, have come from these cells to circulate in the blood stream. By digesting protein A the cell comes to produce more protein A-digesting enzyme. Yet these enzymes or antibodies themselves are specific proteins. Here, then, is a bona fide case of specific synthesis.

Other quite comparable cases exist in which the presence in the fluid around the cell of some substance on which the cell must act, somehow leads to the appearance within that cell of the appropriate enzyme. Thus, yeast or bacteria normally metabolizing one substrate and unable to attack some related one may acquire this second talent. Ordinary yeast ferments glucose voraciously but will not touch the closely related galactose. This same yeast, kept for days in a solution with galactose but no glucose, comes to ferment the latter, and enzymes extracted from it do the same. Bacteria grown under conditions of poor oxygen supply may greatly increase their content of cytochrome and other oxygen-carrying substances, just as man, kept under low oxygen, increases the hemoglobin in his blood. In the case of these unicellular animals it is not now highly important to us whether the new enzymes are synthesized by the original cells or whether, in the course of reproduction of one generation after another, there is a type of natural selection occurring so that "fit" individuals are selected to form the final population.

In all of these examples the substance synthesized is a specific protein with many properties of an enzyme, foreign to the normal cell composition, and appearing as a response to the presence of the substance upon which it acts. The process is related to that of autocatalysis. In the latter, the reaction catalyzed by the enzyme leads to the production of more of

the same enzyme; in the former, to some other one. Yet neither reaction can go forward without substrate of some kind and, if this were initially deficient, its addition would lead to the production of more enzyme, exactly as in the present cases. In any event, such autocatalytic systems clearly underlie all the phenomena of growth and repair and are even more intimately related to the problem of specific synthesis.

When any cell or organ is allowed to exercise its proper function it tends to become more efficient and actually to grow anatomically. This is particularly well seen in the effect of training on muscles. The athlete develops more power, endurance, speed, and coordination in his movements and his muscles become larger and more firm. This cellular growth or hypertrophy involves synthesis, for the cells have not merely become swollen with water or saline solution but actually contain more glycogen, phosphate compounds, myoglobin, ascorbic acid, glutathione, creatine, and the important catalytic systems controlling their metabolic activity. Of course, in terms of purpose, the utility of such internal responses to functioning is obvious enough; indeed, it is a biological verification of those unpleasant precepts with which we have all grown up: "learn by doing," "practice makes perfect," "as a twig is bent so grows the tree" and, perhaps most apposite of all, "the fat boy gets the pie." This principle of growth by use is one of the most general and important met throughout the whole living world, though rarely seen in terms so simple and direct.

But looked at in terms of mechanism, such examples are again a problem of specific synthesis; for in these, even more than in the preceding group, there is an increased formation of complex and important substances which are entirely normal to and characteristic of the original cell. If repeated muscle contraction leads secondarily to an increase of the normal muscle constituents, perhaps entirely similar processes are responsible for the original synthesis of these substances. In one or two simplified cases, at least, this can be shown to be true and the whole chemical mechanism elucidated.

During contraction, it will be recalled, hexose-phosphates are acted upon by the enzymes to form, through a large series of intermediates, lactic acid. Some of the intermediates plus other substances, particularly inorganic phosphate, combine during these same reactions to give more hexose-phosphate. Appropriate enzyme extracts of the muscle, with the right substrate present, will form lactic acid only after hexose-phosphate is added. In its absence no reaction occurs; in its presence, not only is the lactic acid rapidly produced but more hexose-phosphate accumulates as well. Here, then, is a clear case of autocatalysis and of specific synthesis, not of an enzyme but of an important substrate.

The Process of Manufacture

Of course it follows from the very nature of catalysis that every enzyme which splits proteins or sugars or any other cell food or constituent to simpler products can equally well bring about the combination of these simpler substances into the more complex one. In the case of sugar, this oscillation between simple and complex is clearly present under ordinary conditions. Cells take glucose or lactic acid from the blood and build them into glycogen, only to use this polysaccharide store for the subsequent production of glucose and lactic acid. True, the further breakdown of sugars to carbon dioxide and water does not seem to be reversible in the animal, but in the plant even this part of the cycle is completed. (It is not true, though, that the same chemical steps and enzymes are involved in the photosynthesis of sugar and in its oxidation during respiration.)

One condition making for synthesis is the absence of oxygen. We saw earlier that substrate molecules can be oxidized by oxygen, which produces much energy, or by other carbon compounds, which yield minor amounts of energy. The oxygen is reduced in such reactions to unimportant water, but the organic molecules so reduced become the supply for

further synthesis. Just as catabolism involves the breakdown of larger molecules by oxidation, so anabolism emphasizes their synthesis by reduction. Plants burn sugar for energy but rebuild it, during photosynthesis, by a reduction of carbon dioxide and actually release free oxygen. A further reduction of sugar, by plants or animals, is required for building it into fats.

Plants build proteins and other nitrogen-containing compounds with the nitrogen in the reduced, or ammonia, form but cannot use ammonia in the soil for this synthesis. Rather oxidized nitrogen, nitrates, must be supplied and these be reduced by the plant as part of the manufacturing process. An analogous situation in animals has been examined earlier. The amino acid alanine does not form by a combination of ammonia and lactic acid, which would be the most direct reaction; but only by adding hydrogen to pyruvic acid in the presence of ammonia, the synthesis occurring in the course of the reduction.

A decreased oxygen supply may thus favor anabolism and growth. Perhaps the hypertrophy which follows activity of an organ is helped by the partial asphyxia produced by the great increase in oxygen usage. Perhaps the partially anaerobic conditions under which fertilized eggs develop into embryos encourage their intense synthetic activity. At least it is interesting that many reduced sulphur compounds, and not their oxidized alter egos, seem to stimulate growth and regeneration.

The mechanism of protein synthesis is far less clear. Each cell must, of course, make its own unique proteins and make them from the amino acids in its environment, brought by the blood stream or produced by the digestive action of excreted enzymes. The kinds and amounts of amino acids depend, however, rather on the proteins that have been previously digested than on the characteristics of those to be built. The blood, like a cafeteria, offers the same culinary wares to all its customers, yet the muscle and the liver and the brain cells take from the counter such materials as they separately need to build their individual protoplasms. Either the cell membrane allows only
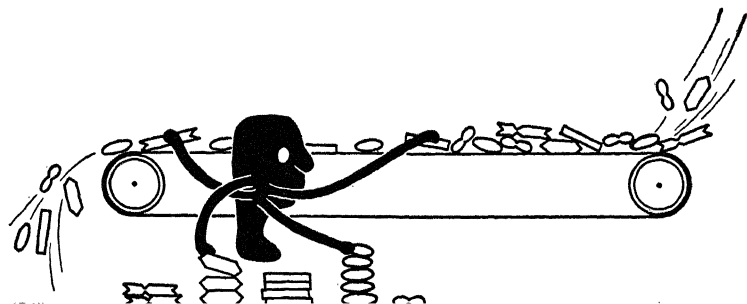
Fig. 134. Every cell somehow takes, from a common supply in the passing blood, the proper amount of each kind of amino acid to form its own unique type of protein.

the right amounts of the right materials to enter, against which suggestion all the evidences of experiment protest forcibly, or else within the cell itself each of the synthetic enzymes, to change the metaphore, performs its own proper prestidigitation and draws the right number of the proper kind of amino acids into its catalytic hat.

If a particular enzyme, or group of enzymes, in liver cells is able to make liver protein from a general pabulum of amino acids, then similarly this enzyme should be able specifically to break down the liver proteins. This is true, for every cell can, under appropriate conditions, digest itself or autolyze. Cells killed by mechanical injury, as following freezing and thawing, or kept in the absence of oxygen and food until they asphyxiate or starve to death, and kept sterile so that the appearance of a new group of bacterial enzymes does not complicate the picture, undergo autolysis. That this depends on the action of enzymes in the cells is shown simply by heating them to 70°C. for a few minutes to kill these catalysts. Such cells retain indefinitely the structure and composition they possess at the end of heating. They do not liquefy or putrify, but remain as wet mummies of themselves. If the enzymes are left, however, the whole cell mass is digested and liquefied and, among other changes, the proteins are split to their constituent amino acids.

The question promptly arises: what determines whether the protein-manipulating enzymes of a cell act to produce synthesis and growth or breakdown and autolysis? One factor is the supply of amino acids. Since the enzymes catalyze the reaction, amino acids⟷proteins, in both directions, a large supply of amino acids favors the production of protein; their absence, its breakdown. This appears clearly enough in cells and organisms—they grow when well fed and waste away during starvation. But other factors are also important, for the ordinary body cells will eventually autolyze if oxygen is withheld, even though they receive ample supplies of amino acids. It has been shown, in fact, that certain of the proteolytic enzymes of cells are inert in the presence of oxygen but become powerfully active in its absence. This activation depends on several changes, including an actual oxidation or reduction of the
e molecule itself. These enzymes contain the sulphur-
  combination of glutathione which, when oxidized, joins
  ilphurs together to make an inert molecule but, when
  d, adds a hydrogen to each sulphur and splits into two
  te halves, each potent.
  clearest possible case of specific protein synthesis has
  y been worked out in some detail. The viruses have
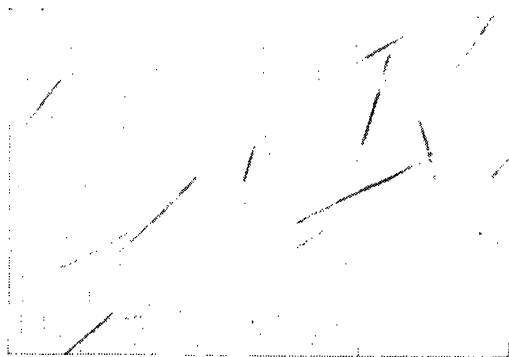
Microphotograph of crystals of tobacco mosaic virus (magnified 700
(Photograph by courtesy of W. M. Stanley.)

long been known to show the property of autocatalysis, or of growth, as it was respectively called by those who considered a virus to be a non-living enzyme or a living organism. These agents are identifiable in terms of their ability to produce change, disease, in other living organisms. They multiply only while acting upon a living organism. In these respects, a virus behaves like a bacterium producing an infectious disease. A tobacco plant infected with the tobacco mosaic virus develops a characteristic mottled or mosaic appearance of its leaves. The juice from such an infected plant, when added to another, causes a repetition of the diseased state. It is now established that the agent involved is nothing more or less than a specific protein, and molecules of it have been crystallized in highly pure form. When added to a tobacco plant, these crystals produce the disease.

It happens that these virus proteins are giant molecules, even in the protein family; but, though this was of great aid in their isolation and purification, it is quite incidental to the point at issue. The important fact is that addition of this foreign and entirely individual protein to the cells of the tobacco plant leads to the formation of more of this protein. Without the living cells, such protein molecules remain as inert as any other reputable chemical. With them, the virus molecules with their aid, or the cells under the viruses' influence, depending on one's point of view, proceed to reduplicate this protein. The multiplication process continues until a large percentage of the protein in the plant is virus protein rather than tobacco protein, and the plant is seriously injured. Here, then, is specific protein synthesis and autocatalysis in almost diagrammatic form, and an ideal case for further study of the process.

The discussion so far has, perhaps, yielded some insight into the problem of specific synthesis and the cell mechanisms for solving it, yet has not really touched some central issues. If cells can and do manufacture the most elaborate sort of chemical molecules, why are certain ones, relatively simple

chemically yet of the utmost importance to health or even survival, quite beyond their synthetic powers? Certain amino acids can be manufactured by animals; others, almost identical and equally essential to the formation of their proteins, cannot be made but must be eaten in the food. One amino acid, tyrosine, is present in all cell proteins; but only the thyroid gland cells can modify the molecule in certain ways and combine it with iodine to produce the thyroid hormone. Yet this hormone is essential to the well-being of nearly all body cells and must be continually supplied to them through the blood.

Large numbers of sugars and related molecules are built and destroyed continuously and with the greatest facility by almost every cell in the animal body; yet one substance, ascorbic acid, which presents the chemist less of a manufacturing problem than do its cousins in the sugar groups, cannot be made by most animal cells. But ascorbic acid is highly important to their health and function. It is·vitamin C, which prevents scurvy; and in its absence, teeth fall out, gums and bones bleed, blood cells become abnormal and fewer, and eventually death results. Even an advanced case of the disease is healed almost miraculously by a few milligrams of this vitamin, which the body needs but cannot make.

No answer is in sight to the question, why are there such things as vitamins—substances which are essential to healthy life and which function in minute amounts, yet which cannot be synthesized by the organism which needs them and must be taken in the food, prefabricated by some other organism—except the limited one that these are present in the foods taken under natural conditions and therefore no necessity arose to make them. The plants, of course, are not so "hothouse," despite common belief, for they, at least the green ones, make everything that they need, and that the animals need as well, from plain old soda water, ground salts, and sunshine.

Finally, the real mechanism of synthesis, at least of the all-important proteins, remains a challenging enigma. Enzymes,

supply of substrates, chemical change sequences, and the like are the magicians' paraphernalia which we recognize, but the cell's hand is still quicker than our eye. Just think what must happen to build some individual protein, with the same hundreds of amino acids as in millions of others but uniquely identified by the molecular pattern into which they are embroidered. Whether in chains or plates or hollow spheres or prisms, each position in the lattice must be occupied by the right amino acid. To revert to the old metaphor, the proper letters must be in the correct positions to give particular words and sentences. A plentiful supply of chemical type is available, but how do the cell enzymes, acting mechanically and without insight, perform the intelligent function of the human typesetter?

Yes, it is not difficult to obtain enzymes which will combine two amino acids to a dipeptide or many into a polypeptide or even into a simple protein; but in these cases the product of synthesis is helter-skelter. Whatever amino acids are present become combined in varying order and number to give a chaotic array of all the possible products. What chemical magic is used by the cell enzymes properly to set their amino acid type into protein paragraphs which mean definitely "liver" or "brain" or "muscle"?

Since spatial arrangement within the protein molecule is an essential part of its identity, it is almost certain that spatial patterns must give important aid in its manufacture. This can be crudely visualized, for example, in the autocatalytic production of one protein molecule by another. In the original molecule the amino acids are, of course, properly located. If each acid were able to adsorb upon its surface a second amino acid molecule of the same kind, a reasonable enough assumption, the adsorbed amino acids would automatically be arranged in the proper pattern. Indeed, this is how all crystals grow, the proper molecules entering the proper places under the influence of intermolecular forces which are highly discriminatory—the one
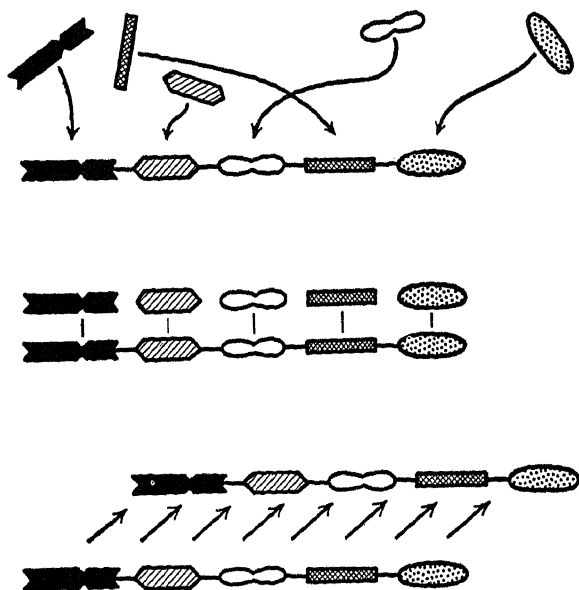
Fig. 136. If each amino acid in a protein molecule adsorbed one like itself, the adsorbed amino acids would be in the proper arrangement. And if they combined together on leaving the adsorbed positions, the original protein would be reproduced.

but not the other of two stereoisomeric molecules, for example, being added. It is the next step that the scientist's eye does not follow; yet we can imagine the adsorbed amino acids being somehow or other simultaneously released over the whole surface and then promptly combining with each other, to form a new protein molecule identical with the original one. A new mold has been struck from the old one. Something of the kind, though not so crudely mechanical, must surely happen. In fact, as we shall see later, it can almost be observed under the microscope, and the powerful new methods of protein study which are coming into being give a bright hope for the future unraveling of this problem.

Chapter Eleven: Oaks from Acorns—
Growth, Reproduction, and Aging

The largest living things to which biological evolution has ever given birth are the majestic sequoias and the "Big Tree of Tule," standing today on the Pacific slopes. The almost fabulous dinosaurs of the past, even the blue whale of the present, have never approached their mass or stature. Bigness is hardly an end in itself, biologically speaking, and far from a guarantee of ultimate success against smaller competitors. The tiny mammals survived the giant reptiles with which they co-existed; perhaps even their very smallness and agility helped them to destroy their powerful but lumbering adversaries by so simple a maneuver as eating the dinosaurs' eggs. Even today, the mouse is a far more successful mammal than is the elephant, which seems to fear it; and the diminutive insects are still regarded by competent entomologists and by tropical explorers, even by summer picknickers, as the most formidable competitors of man.

Yet all living things grow. They grow in size and they grow in complexity. (Growth may be in the negative direction and organisms may grow smaller or simpler, but these are only special instances of the general case.) The value of complexity, or increasing organization, we have seen illustrated over and over and must study it further in the next chapter. Now we can limit our attention to growth of mass. But this much must be
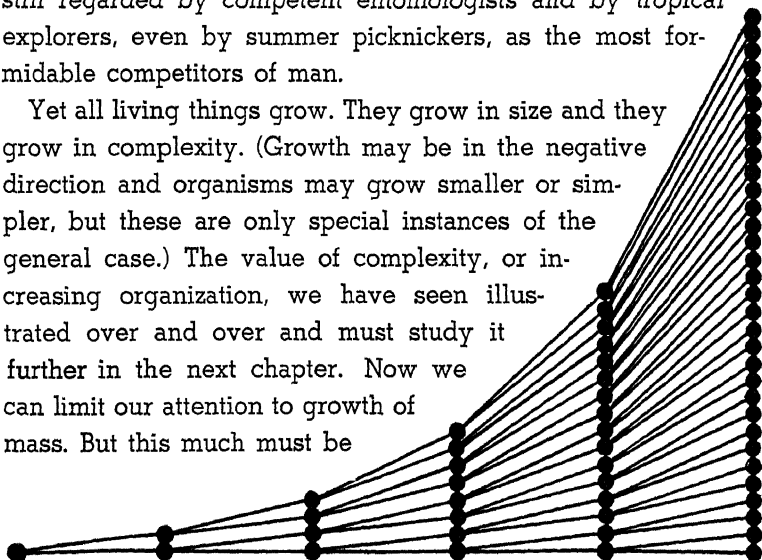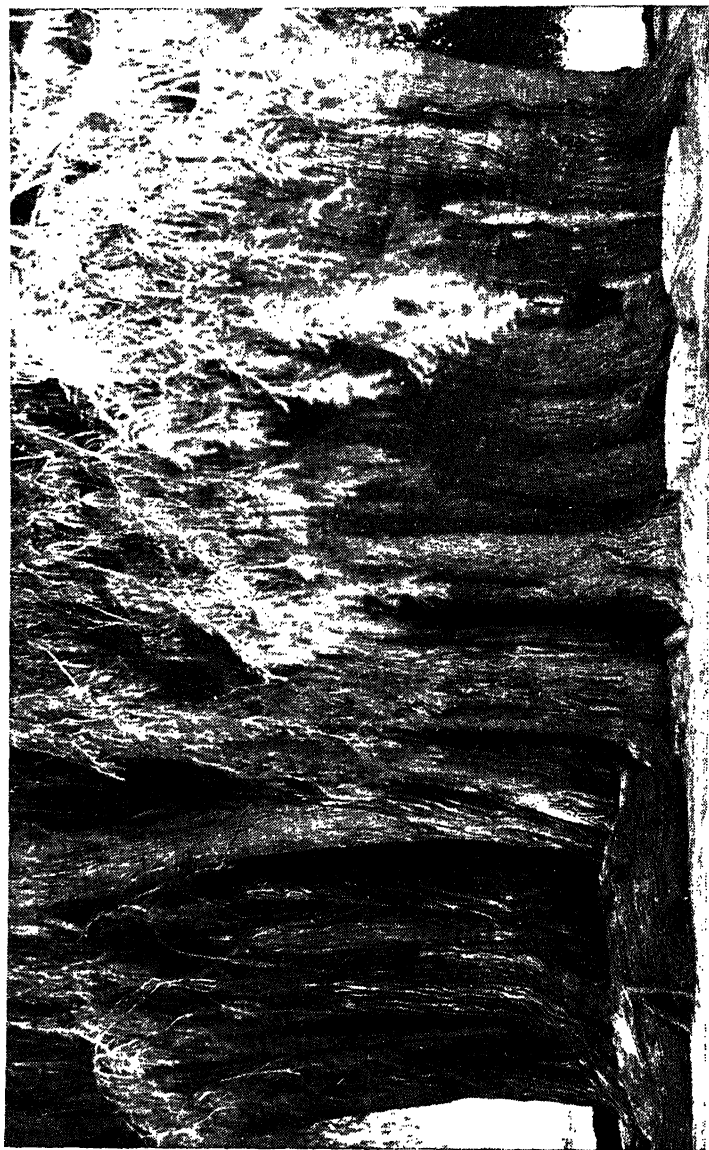
FIG. 137. Base of the giant tree of Tule. The largest living organism. (Photo by F. H. Lamb, courtesy of the American Forestry Association.)

said—growth and differentiation are to some extent alternate processes. Undifferentiated cells grow most vigorously, highly differentiated ones often not at all; when growth in mass is occurring differentiation is usually in abeyance, and the reverse. Plants commonly grow in the dark, rather than when photosynthesis is occurring in the light; and the passage of a cloud across the sun can be registered as a tiny burst of elongation when its shadow falls upon a seedling.

Some increase of living substance between the birth and death of each individual is obviously necessary. Else what would prevent accidental destruction of successive individuals from wiping out a race of cells or larger organisms, and how could living things increase and multiply and spread to every conceivable niche on our globe? For, without growth, reproduction would be merely a splitting into ever more and ever smaller units. An adult man is fifteen billion times as large as the fertilized egg from which he grew. Is so much growth needed? Is it alike for all individuals and cells? When does it stop and what stops it? If great size itself is no object, why is an adult whale 10^{21} times as large as an adult micrococcus? And in what way does this universal attribute of living things differ from those of irritability and metabolism?

Perhaps growth is most set apart by its different relation to time. The ordinary responses to stimuli by animals and their cells flash to completion in thousandths or tenths of a second. The chemical traffic of metabolism moves at a smart pace, with a single enzyme molecule changing as many as one hundred thousand others in a second, and complicated sequences of chemical reactions are completed within a few minutes. But growth is timed in terms of the life span of the individual and unfolds its story in slow motion over hours, days, or years. Of course this is no unique property of life, independent of the others; for the specific and other syntheses which lie at the heart of growth itself are only one aspect of metabolism; and activity, the response to stimulation, commonly leads to in-

creased growth or hypertrophy of the active unit. Rather, growth is the secular trend of the life processes, the accumulated irreversible residue of the fleeting and often reversible events which, moment by moment, constitute the act of living.

Well, then, if growth depends on time will not the length of life determine the amount of growth? But surely this is not true, for man reaches his full height in the teens and his full weight a few years later, barring the obesity of excesses or disease, while his life span carries on another two score and ten. Indeed, if growth continued through life, would an organism ever become physically adult? Yet, continued growth through life does, in fact, hold for a great many living things, perhaps the majority. The limited growth with which we are familiar in ourselves and the common animals that surround us is rather the more specialized condition; the bulk of plants and the simpler animals possess unlimited growth.

It is certainly true that the big fish is usually older than his smaller brother, for age can be determined by annual ridge markings on the scales. But whether this would continue to be true indefinitely is not known, for few fish in nature die of senility and perhaps their growth would have stopped before they lived out their full span of years. Studies in progress on the fish population in a number of midwestern lakes may give

Fig. 138. Unusually old sea anemones are not only much larger than "normal" younger ones, but are also harder, more brittle, and slower in their movements.

a final answer after sufficient years. The case is clearer in other animals. In the marine laboratory at Naples are two sea anemones much larger than any that have ever been found on the Italian shore line. They were transplanted to some rocks in the aquarium over fifty years ago as little slips of things and have led a protected life ever since. They are painfully old and their hardened tentacles move slowly and with difficulty to push food into their mouths, but they still grow, though at an ever-diminishing rate. Such senescent individuals would have long since perished in their natural habitat but, protected, they have become physiologically decrepit while still growing anatomically larger.

This brings us back to the sequoias, for these are not only the largest but the oldest living things. Trees still growing in the California groves stood there mightily at the beginning of the Christian era and the Tule tree flourished while Egypt rose and fell. Variations in their growth rings tell of lush growth or meager survival in a fluctuating California climate, which has been charted by this means back to a millennium before Christ was born. These trees are largest because they have lived longest; the annual size increment is not out of the usual range. They have lived longest not because of any greater inherent vigor of life, though heredity is important in determining their life span and
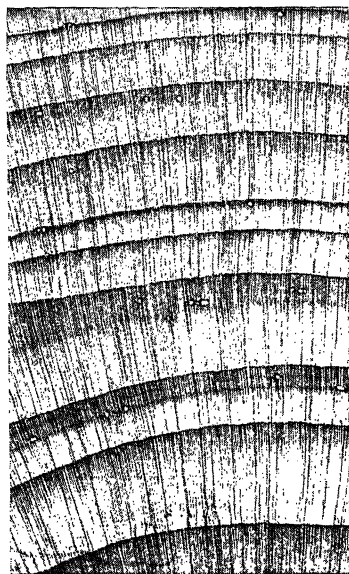
Fig. 139. Microphotograph of part of a section through a spruce tree, enlarged 30 times. The small cells of each growth ring were formed in the fall.

size range, but largely for an almost anticlimactic reason—they are highly resistant to insect, fungus, and virus parasites and simply have not been killed off.

Even the animals with limited growth have a variety of cells which normally display unlimited growth, for example the germ and some blood cells, and many others which can do so under special conditions of handling. The growth of many bones, even when ended in the adult man or dog, can be reinitiated by the presence of an excess of one of the hormones from the pituitary gland. And, of course, the abnormal tumor cells obnoxiously manifest this ability, even in the relatively normal body. The above statements are true only in a broad sense of the term, growth, for in these cases the individual cell does not increase indefinitely in size, but rather it enlarges and divides into two individual cells, which enlarge and divide, in unlimited series. For the single-celled organism, such cell multiplication is, of course, reproduction, and only the size increase between one fission and the next could rigorously be called growth. But in the multicellular animal just such a piling up of separate cells brings about the enlargement of the individual, which in the most rigorous sense is its growth.

Clearly the dividing line between growth and reproduction, like so many others in the living world, is uncertain and broken. Reproduction of cells constitutes growth of the individual; reproduction of individuals gives growth of the community. Indeed, reproduction might be looked upon as a form of discontinuity or a critical point dividing an otherwise continuous growth process into a series of separate quanta. And, conversely, growth of an individual often shows sharp discontinuities akin to reproduction, as in the cases of insects and some vertebrates (frog) which metamorphose strikingly from the larval to the adult forms. Reproduction itself, of cells or individuals, represents an interlude of increasing complexity rather than of increasing mass; for the same amount of protoplasm in two cells is a more complex system than when it is in one. The simpler forms of reproduction, the division and separation of cells, commonly but not always with mitosis, are merely such
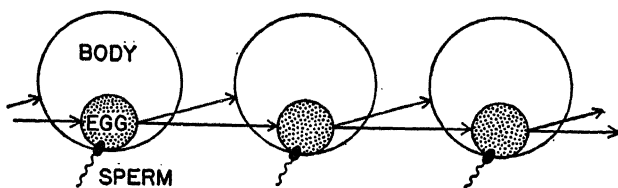
FIG. 140. The egg, when fertilized by a sperm, divides to produce a new egg and the body of a new individual which contains that egg. The egg of each generation is thus a direct descendant of the preceding egg.

a punctuated protoplasmic increase. More complex methods—depending on spores and, still more, on cells of opposite sex which must join together, the gametes—involve further differentiation between the periods of growth.

Thus, in man, the sex cells join and produce a new individual, including new germ cells directly descended from the original ones. These can repeat the process indefinitely, so that gametes normally show unlimited growth. A number of other cell types, in the extragerminal or somatic part of the body, may also continue to grow and divide throughout the life of the individual. The blood-forming cells steadily replenish the blood corpuscles; and those at the base of the skin and of each hair exhibit unlimited growth. In fact, these individual cells continue to grow under conditions that can support their life, whether the rest of the body is dead or not; and corpses buried clean-shaven have shown a growth of hair when later exhumed. A great variety of even the more specialized body cells can survive and reproduce far beyond their normal life span when kept in tissue culture. Like the anemones, they continue to grow when protected.

The Curve of Increase

That growth and reproduction are so nearly the same is shown also in quantitative fashion when one compares the "growth" of cells, cell aggregates, organisms, and populations of single- or many-celled individuals, or even the regeneration of a lost part, such as an amputated tadpole tail. If one plots
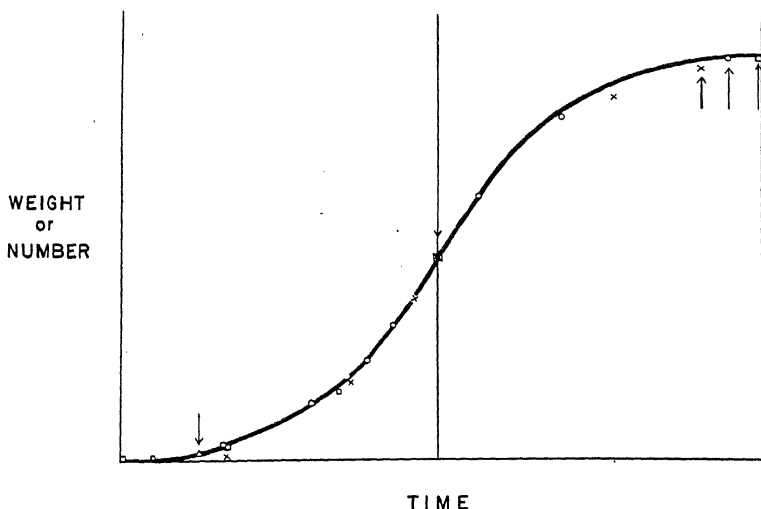
TIME

FIG. 141. The growth, in weight of one individual or in number of individuals, with time is commonly a symmetrical S-shaped curve. When units of time (and of growth) are properly chosen, these curves all become one. Here are shown actual data on the increase in weight of rats (x), from conception to 7 months ($\overset{x}{\wedge}$); increase in number of yeast cells (o), from the time of seeding a fresh nutrient solution to 15 days later ($\overset{o}{\wedge}$); increase in number of flies (□), from the time of introducing one pair into a bottle with food, to 36 days later ($\overset{\square}{\wedge}$); and increase in the population of the United States (△) from 1718 ($\overset{\triangle}{\wedge}$) to 1918 (↓ middle of the curve). All the sets of data exactly coincide at the middle point of the curve. (From several sources, but mainly after Pearl.)

the increase of either number or mass with the passage of time, the resulting "growth curves" are highly similar in these various cases. The population of the United States or France, decade by decade; the weight or height of a man, year by year; the number of yeast cells in a suspension or the size of bacterial colonies on an agar plate, hour by hour; and the length of a single bacillus, minute by minute—all of these increase along the same shaped curve.

This curve starts to rise very slowly, accelerates more and more, passes through a stage of a maximal constant rate, and then progressively decelerates, until some approximately con-

stant maximum is reached. Of course the scale of time and the number of units of increase vary enormously from one case to another, but if appropriate units be chosen for drawing the curves they are practically superimposable for all kinds of growth. Such an S-shaped or sigmoid curve is also met in the process of learning when, for example, the percentage learned is plotted against the number of trials or the time during which learning progressed; and in a very real sense learning is a type of growth at the mental level. Still more impressive is the fact that such a sigmoid curve also documents autocatalytic processes, of which growth, through specific synthesis, is one variety.

Several highly interesting inferences can be made from a consideration of individual growth curves. With the general contour of the curve known, for example, it is possible to extrapolate from one measured portion to another unmeasured one. After half a dozen census records, a part of the population growth curve for a country is at hand; and more or less probable predictions can be made as to the future course of population increase and the time and number at which it will become stationary. A maximum population of two hundred million to be reached by the year 2300 A.D. has so been predicted for the United States. Other completely independent studies, as of the total food resources, have yielded similar estimates of the population this land can support, but it must be added that still others are considerably lower.

Or, turning to man, we see easily that adult dimensions might be predicted early in life. One rule is that the height of a child two years and eight months old will be just doubled at maturity. There is also a suggestion from human curves that man's true life span is almost twice the biblical three score and ten; that, aside from accident or disease, the human machine should run about a century and a half. This is a rather daring extrapolation to make in view of the extreme rarity of even a centenarian, yet even here there is some independent support.

The human eye, for example, decreases its ability to focus near objects as it gets older. This loss of power also follows a smooth course, which is closely similar in all normal individuals, and which extrapolates to zero at the age of 150 years.

But of most interest are the changes that can be produced in the normal growth curve by known conditions, and the light such changes throw on the mechanism of limited or unlimited growth. The early part of the curve, while it is accelerating, is characteristic of unlimited increase and obeys the compound-interest law. Consider, for example, the total mass of bacterial protein in a nutrient medium. One individual is introduced; in a third or half hour two equally developed descendants are present and the protein has been increased by one unit. After a second equal time interval the population is again doubled and the protein increase is two units. In the next interval the increase is four units, then eight, and so on in geometrical progression. Ordinary measurement would detect no change for many hours but then the accumulation of bacterial protein could be followed and, over succeeding hours, seen to form with increasing acceleration. This, then, is the first part of the growth curve.

The question next arises, what converts this spreading explosion of bacterial individuals into a fading and finally failing growth? Is it some intrinsic senility of the individual or race with a shriveling of the growth potency of youth, or is some external factor preventing the continued manifestation of an unimpaired growth power? It is simple to prove the second of these alternates by placing one or a few of the bacteria from the stationary "old" population into a flask of fresh medium. In the new environment the rapid and increasing growth begins all over again and the whole curve is accurately repeated. Some change in the environment, then, rather than in the organism, is responsible for the latter part of the curve with limited growth to some determined maximum by a fixed time. In this case it is clear enough also how the environment limits

growth. The original excess of food in the flask is used up, the waste products of metabolism accumulate, and the microcosm drifts to a static condition with a set or even a decreasing number of bacteria, in a dormant state. Some growth of new individuals and death of old ones probably continue just as anabolism and catabolism do in an adult individual, but the whole remains in sluggish equilibrium.

Does a similar situation hold for a complex individual body? Is its limited growth, or more precisely the limited multiplication of its individual cells, also a matter of environmental limitation? In this case it is clearly not the environment of the animal that comes into question but the environment of the cells, the *milieu intérieur*. If so, there must be some progressive change of the body in time, for the organism—its fluids, nerves, surfaces, and activities—is the environment of its cells. There is much reason to believe that this is the situation and that the limited growth of an individual is determined by the residual effects of the preceding growth itself.

Note that, for the bacterial culture, it was not the passage of time which caused the decline of the growth curve, but rather the growth already consummated relative to the total amount possible. If, at some early stage in development, a culture is taken from the incubator and placed in the ice box, the bacterial protein will remain constant over hours or days. When, much later, the whole is returned to the higher temperature, the original rate of increase is promptly resumed. A comparable situation can be studied in complex animals. In rats, for example, growth is most rapid between six and twelve weeks and is essentially complete at 200 days. A four-week-old rat placed on a diet lacking some ingredient essential to growth, such as certain amino acids or proper salts, but containing all the essentials for life, will maintain the same weight for many months. The animal is now chronologically beyond its growing period, even very ancient; yet if the missing substance be supplied, growth is promptly resumed. The curve,
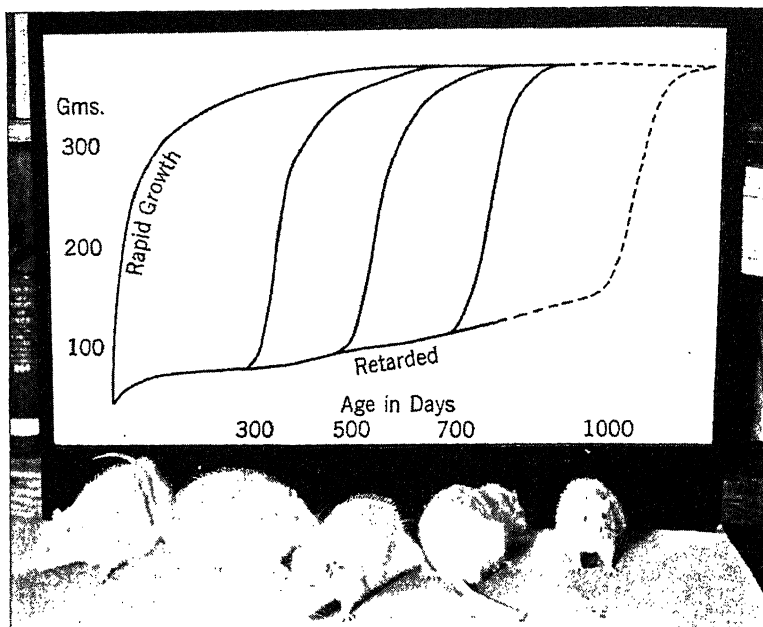
Fig. 142. Growth curves of a normal rat (rapid growth), and of its four litter mates (retarded), kept for varying times beyond the normal growth period on a faulty diet and then placed on an adequate one. The rats, shown below the curves, are now all of normal size except the last, still on the poor diet. (Photograph by courtesy of C. M. McCay. From Cowdry's *Problems of Aging*, The Williams & Wilkins Company.)

interrupted in youth, is completed as if it had never been broken, at the same rate and to the same final level. It is the act of growth rather than endurance in time that finally stops the growth process.

On Growing Old

The existence of such a progressive change with age brings us frankly against the problems of youth, maturity, and senility. That such words have significant meaning in the life of man and his fellow vertebrates needs no belaboring. Pitkin's *Life*
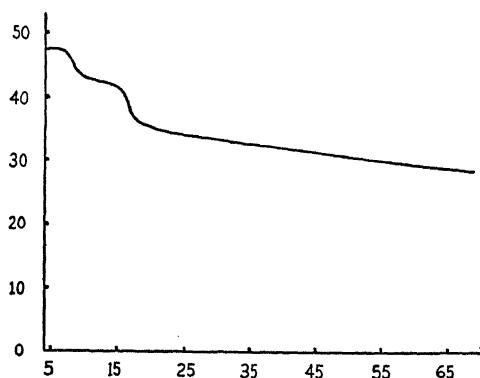
FIG. 143. Curve showing the decrease of basal metabolic rate from the age of 5 years to the age of 65 years. The hump in the fall occurs at adolescence.

Begins at Forty has had such success because aging men, like drowning ones, grasp at straws. Everyone who has reached middle age recognizes in brain and muscle that something is leaving him and, in his heart, echoes Byron's lament, "O talk not to me of a name great in story; the days of our youth are the days of our glory."

A more objective examination fully supports this subjective impression. Athletic champions fade out some time in their twenties, in the earlier part of the decade in those sports in which success depends most completely on strength or speed. The reaction time between stimulus and response progressively lengthens, the resulting movement is slower and develops less total force, the general level of bodily activity falls. These are seen most directly in the gradual decline of basal metabolism after the first two or three years of life, and in the decrease of spontaneous movement, measured in a rotating cage, indulged in during a day by an aging rat.

The mental functions are slowed later and more gradually and it is harder to separate accumulated knowledge and judgment, which favor superior performance, from lessened learning capacity and intelligence attributes, which act in the other

direction. There is some evidence that men in the forties and fifties show undiminished learning capacity when tested under the same favorable conditions as obtain in earlier years; but sooner or later the same decadence is manifest in the brain as in the muscle. Other organs also show the effects of aging, and at fairly characteristic times. The physician can, on such a basis, definitely exclude certain possible derangements when diagnosing an ailment. From middle age onward, symptoms tend to arise from the "degenerative diseases" of heart, blood vessels, kidneys, and intestinal tract rather than from the acute infections of earlier life.

That aging affects the cells themselves is also clearly seen in the process of wound repair. A cut or abrasion in a child may heal completely in a few days and leave no detectable scar. The epithelial cells of young skin, as well as the fibrous cells of young connective tissue, grow rapidly to bridge over the injury. In older people, similar wounds heal slowly and imperfectly, and the growth of skin is even more retarded than that of connective tissue so that a fibrous scar eventually results. Studies have been made of the rate at which new cells spread from the margin of a large injury, say a burn, toward the center, and this also clearly diminishes with age. A bone fracture in an infant is of little moment and a few weeks suffice to knit the fragments more strongly than ever. In the aged, recovery is a long, tedious, and all-too-often incomplete process, because of the slow and limited response of the bone-forming cells.

The aging of cells is shown in still other ways. Not only do
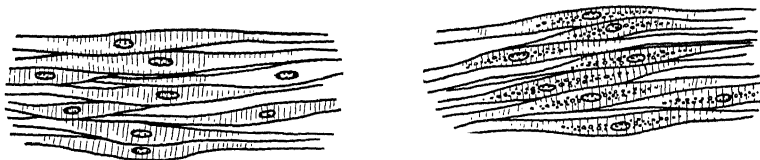
FIG. 144. Granules of various inactive materials, often pigmented, gradually accumulate in cells as they age. The heart muscle cells on the left are from a young animal; those on the right, from an old one.

old bones heal slowly but they also break easily. This is due to an increase in their content of calcium and other mineral salts, and a decrease in their resilient organic material—both of which make them more brittle. All cells accumulate more solids as they age; that is, in a given volume is found a lower percentage of water and a larger amount of residue. Some of this can be seen under the microscope in the form of pigment, lipin and other granules, seemingly debris accumulated in the course of life and progressively cluttering up the cell household.

How, then, can we say that cells age because of a change in their environment, when they themselves are so altered by the passage of time? Comparable changes can be seen in bacteria from old cultures; yet when growth is resumed in a new medium, the active cells are as fresh and watery as their distant ancestors in the original young culture. In the same way, it is impossible to tell by the appearance or behavior of a growing colony of cells in tissue culture whether the original settlers came from a young or an old individual. There is an early difference, however, for the cells from an infant start growing at a good rate almost at once while those from a senile animal may slowly accelerate their growth and division for many days before they attain this same rate. The older cells are, to be sure, aged while in the old body; but since they do rejuvenate fairly quickly it is equally clear that this is no irreversible change in the cell itself. Most metazoan body cells, like their germ cells and like unicellular animals and the bulk of plants, are potentially immortal. The converse experiment shows even more clearly that it is the organism as a whole which grows old.

A culture of young cells, growing well in the artificial medium of a tissue culture experiment, is divided into two and the growth of each portion then followed in a different culture medium. Here the cells are alike and any difference in growth rate must depend on a difference in their environments. Chicken serum, a common constituent of the medium, is obtained in one case from an old bird, in the other from a young
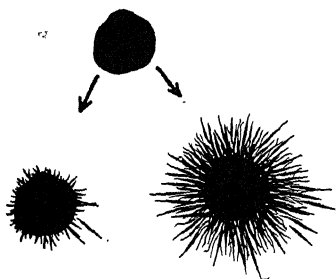
Fig. 145. A bit of cultured tissue, kept in a culture medium containing serum from an old chicken, will appear in a few days as shown on the left. If kept with "young" serum in the medium for the same period, it will appear as on the right, having grown much more vigorously.

chick, all else being kept identical. The half-portion of cells in old serum grows slowly, that in young serum grows rapidly. Here, then, is a quantitative index of aging of the cell environment in the intact animal. Old blood serum imposes on the cells it bathes the character and behavior of age.

Of course, such a statement is merely a challenge to the experimenter to define in more material terms the difference between old and young sera. Age is not some abstract essence that accumulates in the blood as the animal lives on, but is simply the increase or decrease in amount of particular substances. What these are is not yet fully known; but the serum lipins are more concentrated in older animals, and lipins extracted from old serum and added to young serum give the latter "old" properties. Cells, then, age in the environment of an old body; but this environment must, in turn, be produced by old cells. Something is still eluding our analysis. Where are the lipins of old serum formed and, more important, what has happened to the cells in an old animal that they should form them? To answer that these cells have aged and, therefore, have produced the age substances, takes us right around a circle.

The structure and function of an organism have often been compared to a river bed and a river, and many shrewd analogies exist between the stream of life and that of water. One cannot say simply that the banks control the flow or the stream its channel, for each acts upon the other and reacts in turn upon itself. When a river gets old it also shows a slowing of function and characteristic changes in structure; yet the

water is not older, nor the sand and mud, but only the whole system or organism. Note, further, that aging here also is not a matter of time but of process; a river which is dry most of the year ages more slowly than one with a continuous stream. And rivers can also be rejuvenated by some geological catastrophe. In this case the mechanism is clear. Aging is the result of a loss of slope which decreases the gravitational flow; rejuvenation follows a reelevation.

In the same way, rejuvenation of cells or organisms depends upon the interaction of the unit and its environment and on some sort of biological cataclysm. Rejuvenation in a culture is ordinarily not of the particular old cells introduced but of their descendants, born after repeated cell division. There is much reason to believe that the extensive and dramatic rearrangement of protoplasm attending division is indeed a rejuvenating experience for the cell. Whether this is a consequence of increased metabolism, due to the freeing and mixing of catalysts and substrates and the consequent burning away of the accumulated cell debris; whether some reserve stores, special cell parts or cells divorced from the wear of daily life, are then tapped; or whether some still unguessed processes are at work, we cannot say. Certainly the combination of egg and sperm (even the appropriate stimulation of the egg alone, in parthenogenesis), following which similar but more spectacular cellular revolutions unfold themselves, leads to striking rebirth of their protoplasms.

In fact, many protozoa which can reproduce by simple mitosis, normally supplement this process at intervals of several generations by that of conjugation, a primitive type of sex act in which nuclear materials of two individuals are exchanged and mixed. Following such exchange, or even a special reshuffling within one animal, there is an increase in general cell vigor, with rapid growth by mitotic multiplication. Even in the more complex organisms catastrophic rejuvenation can occur. If aged flatworms are starved, so that they metabolize their
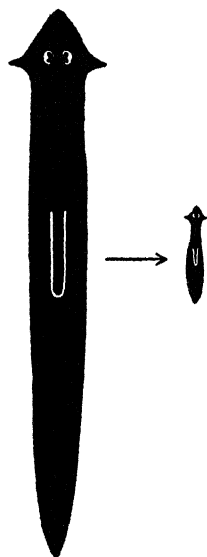
FIG. 146. The "old" flatworm, on being starved, shrinks to become the "young" rejuvenated animal. (After Child.)

own substance, their size shrinks considerably. These starved animals, when again permitted to feed and live normally, behave like young individuals which have just attained that small size. They again grow and age and can be again starved and rejuvenated. Individuals have actually been kept alive by such handling for periods longer than the normal life span of twenty generations.

Rejuvenation and aging, alternate and successive processes, might recur at shorter and shorter intervals until finally a steady balance between them resulted. Animals fed and starved over long intervals, like the annual hibernators, such as the bear, follow a zigzag course between fat and thin; those which eat regularly, with but brief interludes between small meals, maintain an even weight. Age and youth might similarly be in equilibrium if the changes of decadence and rejuvenation were shuffled frequently enough, and an individual could endure, even grow, at its normal metabolic pace, without becoming physiologically older.

A cell culture, transferred often to a fresh medium, grows in perpetual youth. A plant cutting, which normally would remain part of the original individual and die with it, can grow into a new plant, its cutting into another, and on indefinitely. An annual, which normally seeds—and then dies—in a year, can be kept from flowering—and dying—by control of illumination; it then carries on a healthy vegetative existence as long as the experimenter's patience demands this. Even within the single cell, growth can be maintained, apparently indefinitely, without aging or cell division. A single amoeba has been kept

growing and healthy for four months by the simple expedient of cutting a chunk from it whenever its size increased toward that at which it would normally divide.

On the other hand, even some protozoa die a "natural" death. Instead of simply dividing their total substance into two daughters, they encyst and only part of their protoplasm becomes the next generation. Some insects, after metamorphosing from a voraciously feeding larva, become imagos (adults) which completely lack a digestive apparatus and are thus doomed to early death by starvation. Vertebrates seem to have hardly less fixed normal life spans—extremely few as long as fifty years, the great majority under fifteen. And the giant sequoias, alive three or four thousand years, may no less surely be pointed to a distant end, for their annual growth rings are becoming narrower century by century. These cases are ones in which rejuvenation has not naturally or experimentally been kept in pace with aging. But protoplasm itself does not run down; for living things or parts of them, in whatever species, have continued to exist and grow with undiminished vigor since their origin in the distant past and will presumably continue to do so.

Is retaining or regaining of youth possible with man? Perhaps in the future, though not by so simple a device as interrupted starvation periods. Nor have the much heralded effects of testicular duct ligation or gland or hormone treatments survived the test of careful experimentation. Longevity can be increased in many ways. In a cold-blooded animal, like the fly, lowering the environmental temperature and slowing metabolism increases the life span. Control of accidents and disease has already tripled human life expectation; but only by preventing premature death, not by postponing natural disintegration. Even this, however, could be achieved today with our present knowledge.

Longevity, like height and skin color and so many other physical traits, has been shown to be strongly influenced by

heredity. The difference in normal life span from one species to another is, of course, dependent on inheritance, but even within one species the life expectancy of an individual is largely inborn. One inbred strain of flies averaged eighteen days of life per individual, another strain averaged forty. In the broad sweep, individual persons whose parents and grandparents lived to ripe old ages are likely to do the same, and the surest way of becoming an octogenarian is to make a judicious choice of ancestors. But by what physiological or chemical mechanisms some hereditary factors make for a long-lasting machine and others for one that cannot be given much of a guarantee are problems for the future. Perhaps some time science may penetrate these secrets and restore to metazoa their birthright of immortality, which was exchanged eons ago for a shorter but more elaborate and efficient life. If this day comes, however, man may seriously regret his achievement.
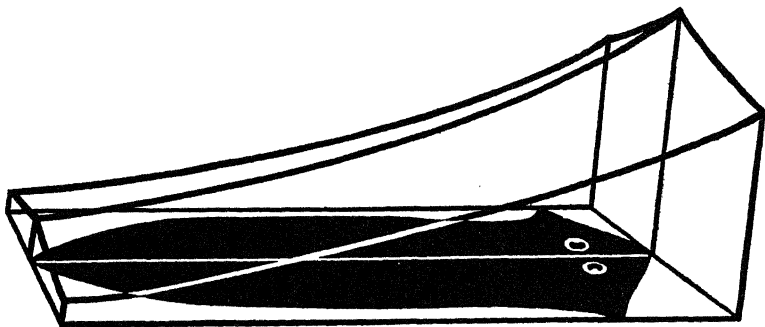
Chapter Twelve: From the General to the Particular—
Differentiation, Embryogeny

It has become trite to say, "The whole is greater than the sum of its parts," but an organization of units *does* have additional properties which are not present in a mere aggregate. Two ones in proper constellation are 11, not merely 2. As we have seen, living things are supreme among the known systems of the universe in the intricacy of their organization. From the atomic level, through those of molecules and colloids, to that of the cell it has been possible to trace the ever-richer design. How it is woven remains still largely a matter of mystery, despite some of the clues that we have followed up. Above the cell level the design continues; indeed, the weaving of cells into tissues and organs and general body form impressed the observant long before the more intimate complexities of protoplasm were remotely suspected. Here, further, is offered a peculiarly happy opportunity to watch organization develop; for, as the microscopic fertilized egg grows into the myriad-celled completely formed organism, its kaleidoscopic changes can surely be observed and perhaps even be experimentally modified.

In principle, the development of a multicellular embryo is simplicity itself. The fertilized egg divides by mitosis, the daughter halves repeat the process, and eventually great num-

bers of cells are formed. Of course, cell growth intervenes between the divisions, at least after a few of the earliest very rapid ones, so that the total mass of the individual increases. This process of growth and multiplication, which we have already examined, gives an adequate bulk to the body. There remain, then, only the problems of: (1) the differentiation of the original cell into thousands of distinctive, often strikingly different, cell types; (2) the number, often billions, of cells of each type; and (3) the spatial arrangement of all these like and unlike cells in every minute part of the final whole. Only these questions, yes, but when they are fully answered there will be no mystery left in life.

Potentiality and Performance

Consider the differentiation of the few original cells, all apparently identical, into the great variety which ultimately appear as their descendants. Brain or liver or muscle cells are all the offspring of the original fertilized egg; and, if like comes from like, are they, perhaps, still essentially the same cells but manifesting different characteristics because of their special conditions of existence? Or has something been lost along the line, or certain of the potentialities of the original cell been divided and parceled out so that each offspring eventually possesses only a portion?

A family fortune can be liquidated and the capital distributed equally among the children, each of whom may then build up his inheritance until the parental magnitude is reattained. Or a fortune may be inherited in distinct units—a house by one child, a business by a second, real estate by a third—in which case it is unlikely, sometimes impossible, that the life work of the offspring will reproduce the ancestral estate.

So in development, the germ cell is toti-potent, its inherited biological capital is fluid and can develop and deploy in any or all of a large number of directions. The question asked above, restated, is: are the specialized offspring cells toti-

potent or have they sacrificed versatility for expertness and, if the latter, to what extent is this choice irrevocable? A particular bràin cell in a man traces its ancestry back to the egg, through hundreds of cell generations. Was some particular bit of the egg protoplasm preordained to this destiny or, if not, at what point along the scheme of division and redivision did a cell appear which was able to produce only nerve cells as its own descendants? Or is it, perhaps, still possible for the fully specialized and developed nerve cell to dedifferentiate and revert to a more generalized type, one which could develop skin or liver or what you will when again given a chance to specialize? Clearly these questions have now become quite precise and well within the range of experimental answer.

Each nerve cell possesses, we have noted, at least one long fine process which may extend for inches or feet. If this be cut across, the isolated portion dies but the remaindeı survives the injury and eventually grows out to reform the amputated segment. The nerve cell, then, is able to repair itself after moderate injury. The regrowth of a bundle of such nerve fibres is the body's way of recovering from section of a nerve, and it results in the ultimate restoration of the sensibility and muscular control which were lost when the nerve was cut. But when a nerve cell is more seriously injured it simply dies. Injuries to the brain, produced by disease or accidents, lead to the death of large clusters of nerve cells and present an opportunity to study whether these can be replaced in any way.

The answer is unequivocally no. The surrounding uninjured nerve cells, in fact, do nothing whatever about the injury. No conditions have yet been found, even in tissue culture, under which fully developed nerve cells can be made to undergo mitosis and to multiply. Nor can these cells "change their spots" and come to resemble some other type by gradual metamorphosis without division. Indeed, these neurones are so fixed that one of them, characteristic of a particular part of the nervous system, cannot even turn into another equally good one, which is characteristic of another position. The adult
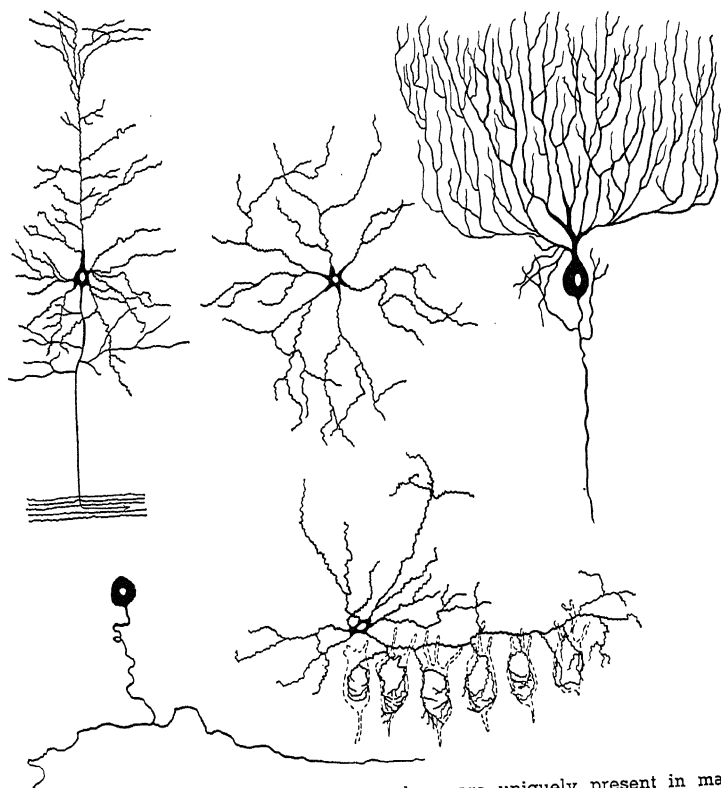
FIG. 147. Nerve cells of characteristic shape are uniquely present in many parts of the central nervous system. One cannot change into another. The pyramidal or Betz cell (upper left) is seen only in that part of the cerebrum which controls voluntary motion; the Purkinje cell (upper right) occurs only in a deep layer of the cerebellum, as does the basket cell (lower right) which connects many bodies of the Purkinje cells. A sensory monopolar cell (lower left) serves only to bring messages into the spinal cord, and internuncial cells (center) are found as connectors in many parts of the nervous system.

neurone is a terminal station with no transfer or return privileges. The change from the antecedent cell to it is final and irrevocable; even the ability to reproduce is lost and only by death is the cell diverted from its continued characteristic existence.

The neurone, then, has clearly lost all potencies except those inherent in being a nerve cell. But is this case general for all cell types? If we look again at the injured brain we see the destroyed area soon replaced by clotted blood. Enmeshed in the clot are the various white cells which formerly circulated. Some of these are the amoeba-like phagocytes which move about in the dead debris and gradually eat and remove much of it. But the most fascinating behavior is shown by another group of these cells, the lymphocytes, ordinarily carried about passively and inconspicuously in the blood stream. These small globular units, composed mainly of a large nucleus surrounded by a thin shell of the most generalized kind of protoplasm, come dramatically into an active life. They grow, start to move about, phagocytize food, divide, move and eat and grow again till relatively large cells result. Each successive generation shows distinct changes from its immediate parent,
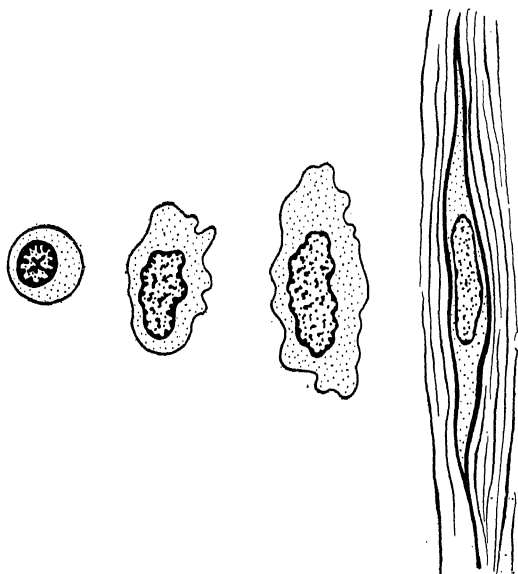
Fig. 148. The round macrophage transforms itself into the elongated fibroblast, which then deposits connective tissue fibers about itself. (After Bloom.)

and after a few divisions the cells formed are structurally about as different from the original lymphocyte as could well be imagined.

The new cells are spindle-shaped with sharply pointed ends; they are many times larger than the lymphocytes; they have a good-sized oval nucleus imbedded in a great mass of cytoplasm; and their cytoplasm manifests many characteristic structural details, including a fine longitudinal fibrillation, which is completely absent in the ancestral type. Eventually they produce masses of wavy fibres which come to lie between, rather than within, the cells that manufactured them. Most interesting of all, these cells and fibres do not constitute some new cell species into which the lymphocytes have contorted themselves but, by every structural and functional test, they are identical with a type of cell which is normally present in the body in large numbers, the connective tissue cell. In the wounded brain this formation of fibroblasts leads ultimately to the production of a connective tissue scar and "healing."

Examined in normal tissue preparations, lymphocytes and fibroblasts can be seen side by side looking as strikingly individual and distinct from each other as any pair of cells in the body. Yet, in sharp contrast to the neurone, the lymphocyte can, even while watched under the microscope in tissue culture, progressively metamorphose into its antithesis. The reverse change also can occur, though less readily. These two cell types, then, are not rugged individualists but retain certain potencies for becoming something else. It follows that some strains of highly differentiated body cells can dedifferentiate and revert to other types, while some cannot. Of course, even in the former case, there is no evidence that toti-potency has been retained. Lymphocytes and fibroblasts and certain other interstitial cells of the body can change into one another but none of them have been made to form heart or kidney or brain or any one of dozens of other individual cell types.

These experiments have all been done with "adult" cells,

either the actual individual ones present in an organism that has completed its formative development or others of some final differentiated type. It would seem reasonable to anticipate that younger, that is more embryonic and undifferentiated, cell types would show increasing lability as they were more immature. In the same way, the fate of human units in the social organism (in distinction to how good an example of the type) is overwhelmingly determined by the course of their individual lives. Whether a child learns to speak French or English, to till the ground or build machinery or treat patients, is a completely open question at birth. The baby is toti-potent, barring distinct abnormality, but the developing child sacrifices one after another of its potentialities while it fulfills the remaining ones. In the organism, similarly, the fertilized germ cell must carry the capacities of giving rise to every one of the cell types which actually form among its many descendants.

Evidence from Embryos and Tumors

Argument by analogy is useful but carries a serious danger of false conclusion. Is there any actual evidence for this undifferentiated toti-potency of the fertilized egg? All the facts we have so far considered could be well explained in another way also; a set of predetermined units are present in this initial cell and need but unfold along set paths. If a thousand kinds of cells are present in the adult organism, why not a thousand cell seeds nicely packed together in the egg, each able to grow and divide and so produce all cells of one kind? Can this be tested by actual experiment with a single fertilized egg? Yes, in several ways. Shall we again be surprised if nature gives both possible answers to our question?

Consider first a frog or a sea-urchin egg. Parts of the protoplasm are heavier, others lighter, yet normally these various bits are scattered throughout the whole cell. If it be centrifuged, the protoplasm is sorted out on the basis of weight, and quite

independently of any "natural" arrangement, with the lighter portion at one pole and the heavier at the other. In the case of the urchin egg, we have seen that sufficiently strong centrifuging will pull the sphere into two half-eggs, one light and one heavy, which will initiate development after fertilization with a sperm. Even the centrifuged whole egg is soon divided by mitosis into cells with abnormal proportions of light and heavy materials; and the half-eggs are unequivocally fractionated.

Each does not receive an equitable sample of everything originally present. If some unit in the egg produces each adult cell type, then surely these units, displaced by the treatment, will be improperly distributed in the daughter cells. Each of the half-eggs formed by centrifuging should be able, therefore, to develop only certain ones of the possible cell types, and even the centrifuged but unsplit egg should cleave into cells with an abnormal quota of these determined seeds and finally form distorted animals. On the other hand if, instead of possessing specific units, the egg cell protoplasm is truly toti-potent, then each part will still be able to form all types of cells, unless the treatment did such damage as to interfere with development. The answer is perfectly straightforward: no matter how the frog's egg is stirred about or the urchin egg separated into fragments, fertilization initiates cell division, development proceeds, and normal animals result with all the right kinds of cells in the proper positions. (The nucleus behaves as a unit in these experiments and the conclusions do not apply to it in the same way. We shall consider it later.)

A somewhat different experiment leads to the same conclusion. The first cleavage of a normal fertilized sea-urchin egg gives symmetrical halves, the plane between them eventually becoming the midline of the body. The descendants of the one cell form the right half of the adult, those of the other, the left half. Suppose these two cells are separated before further development takes place. Then, if each continues along a predetermined path, it will produce a right or left half of the

animal, or at least will develop as far in this direction as is possible before the monstrosity dies. Actually nothing of the kind results; each separated cell gives rise to a complete and symmetrical adult just as if it had been the original fertilized egg. It is clear, then, that in these animal types the egg is truly totipotent and not a collection of labeled cell precursors.

FIG. 149. A snail egg divides asymmetrically with right or left twist; and the shell of the adult animal is correspondingly spiraled. (Modified from Huxley and De-Beer.)

But the same experiments on certain invertebrate eggs, such as snails or segmented worms, give results which are no less decisive in the other direction. If a snail's egg is centrifuged and then fertilized it proceeds to divide and form various differentiated cells. But in what a hodgepodge! Skin cells may be in a clump on the inside of the animal instead of as a sheet on the outside, and the unprotected liver or brain cells may be plastered upon the surface. Further, the snail egg at its third division forms four large and four small cells, the latter not resting squarely on top of the former but placed a little askew to the right or the left. Just as the midplane of the frog is set at a very early stage of development, so does this skewness determine the direction of spiraling of the snail's body and shell. But, in .contrast to the frog, the snail's pattern is fixed at this stage and cannot be reorganized if the cells are disturbed.

There is a faith in science that nature is fundamentally consistent and the scientist rests uneasy when such a situation as the above confronts him. Can these various conflicting findings be reconciled in any way or must our answer remain a shoddy "either this or that"?

If we were to ask the question, "Can a new-born mammal

manage without maternal help?" we should get one answer from the rabbit and the other from the closely related guinea pig. The newly arrived bunny has no hair to keep it warm, cannot walk around, is blind with closed eyelids, and takes only its mother's milk for food. The piglet, on the contrary, lands, so to speak, on its four feet, a small but well-formed replica of its mother; it licks its mussed hair into place; sizes up the world with its wide-open blue eyes, and promptly sets about the important business of finding and nibbling carrots. The reason for the difference is clear enough here; the guinea pig is born at an advanced state of maturity which is achieved by the rabbit only after weeks of extra-uterine existence. Looking backward, we find that, whereas the rabbit is born after only one month of development in its mother, the guinea pig luxuriates in the uterus for over two. The apparent conflict is now resolved to a minor difference in time of birth. Both animals develop from egg to adult along a very similar course and in similar times; one merely happens to leave its birthplace at an earlier age and arrives, normally, as a "premature" baby.

Perhaps a similar time or developmental difference between the frog's egg and that of the snail will likewise resolve the differences in their behavior which are brought out in the above experiments. Actually, the process of formation of the egg from its antecedent cells affords much evidence that this is indeed the case. The ripe snail egg has undergone far more maturation than has the ripe frog egg and is, therefore, more mature and differentiated at the time of fertilization. Presumably, if it could be successfully manipulated at an earlier stage it would behave as does that of the frog.

Neglecting, then, the somewhat arbitrary stages in individual development, we see that all cases manifest a progressive change from undifferentiated toti-potency toward an increased differentiation in certain directions and a diminished potentiality to become anything else. The snail's egg is further along this road at fertilization than is the frog's; the nerve and muscle cells

are further along it at maturity than are those of the blood and connective tissue. And every intermediate stage can be found somewhere along the line. As the embryo grows and develops, a larger fraction of its ever-increasing cell population is in the form of more specialized cells and a smaller proportion in less specialized ones. Now here, now there, in the tiny body, one or another group of units bursts into increased activity, multiplies rapidly, and produces new and more nearly adult cell types.

The complete genealogy of every tissue cell has not been worked out, but in all cases the major steps are clear and in some every ancestor is known, named, and described. As might be expected, the farther back one goes along a cell's family tree the less do the individual ancestors resemble the final product and the more are they like a blob of unspecialized egg protoplasm. The progressive differentiation can thus be followed by direct observation with the aid of a microscope. Examination by physiological and chemical techniques similarly shows a steady increase in proficiency of function and in concentration of important and unique cell constituents. The evidence that there is a simultaneous loss of potentiality to grow and to become something different is more indirect. So far as testing growth is concerned, the ideal experiment would be to take cells at each one of the stages of development, put them in the same favorable environment, and compare their growth rates and powers—much as was done in the tissue culture experiments. Nature has made practically this very test for us.

A cell at almost any developmental level may become cancerous. Just what causes such an individual to deviate from its proper development as part of a cell society and to set off on an anarchical career of unrestrained private initiative is not known. Many very important facts bearing on this question have been accumulated and it is even possible now, by the use of a number of physical or chemical agents, to initiate at will such wayward and untrammeled growth. But how they work remains a problem, and what the particular change is, in metab-

8

17

25

70 or more
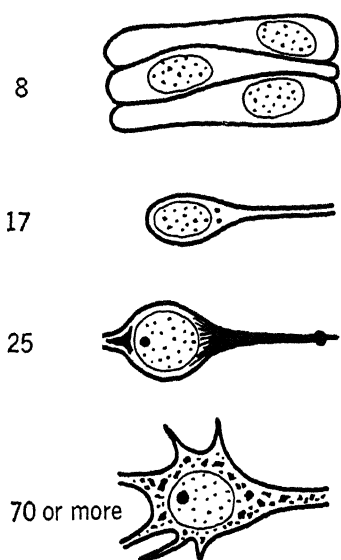
FIG. 150. Four stages in the development of a nerve cell, from the most embryonic condition at the top to the adult neurone at the bottom. Occasionally a brain tumor forms with cells of one of these types; and the more embryonic the kind of cell it contains, the more rapidly does the tumor grow and kill. The number to the left of each cell gives the average months lived by patients after the start of a tumor of that cell type. (After Bailey.)

olism or structure of the aberrant cell, which confers upon it the capacity for excessive growth, is also unsolved. Fortunately these difficulties need not concern us here. The point of interest now is: how do different kinds of cancer cells deploy in similar animal bodies? And the answer is very clear: within a single group, those cancers which derive from less differentiated ancestral cells grow rapidly, spread widely and, over successive cancer cell generations, either remain undifferentiated as they were at the start or else differentiate irregularly into nests of cells of various kinds; those originating from the more differentiated normal cells grow slowly, remain localized, and complete more or less accurately the further development which the normal parental cell would have undergone. The more primitive its cell of origin, the more malignant or fatal is the resulting tumor; the more differentiated its ancestral cell, the more benign or innocuous is the resulting growth.

The successive loss of potency, or the ability to become any one of many final cell types, is shown also by another kind of evidence, which we shall examine again later in more detail. If some of the undifferentiated cells in that portion of the embryo which would normally develop into the digestive tract

are removed by a microoperation and placed near the develop-
ing heart, their further differentiation turns them into perfectly
normal liver cells. These same cells, left in their original place,
would have developed into normal intestine. There is, there-
fore, a time in their history when such cells may develop
equally well into gut or liver and when the direction they will
take is determined by the situation in which they find
themselves.

This experiment would be impossible, of course, before the
embryo had reached a stage of development in which heart
cells and intestine cells are defined. On the other hand, if
development has continued a day or two after these tissues are
defined, and the cells of the digestive system have moved a
little further along their normal differentiation toward gut, the
experiment no longer succeeds. The clump of cells trans-
planted next to the heart is, then, uninfluenced by this proxim-
ity and proceeds, just as if left in its original position, to form
good intestine.

Some Developmental Mechanics

And now comes the really searching question: how is cell
development controlled so that the proper types of cells end up
in the right places and in correct numbers? We can dispose of
the question of number most easily, for there is little knowledge
about it. The number of cells produced *is* controlled, for the
same end result is quite accurately attained time after time.
Further, this number is not preordained any more than are the
kinds of cells, but results from the interaction of developmental
forces. Such a statement as it stands is, of course, meaningless,
but it takes on some significance when we look at the facts.

As an arm bud begins to swell out from the body wall in
the embryo, it is covered by primitive skin, or ectoderm, cells,
and contains connective tissue from part of which bone and
immature muscle cells will develop. Eventually all these

muscles must be supplied by nerves to make them function, but the nerve cells remain gathered in a hollow tube along the back of the trunk. The nerve cell processes, then, must grow down to supply the muscle; and their advance can rather easily be followed microscopically. The normal mature limb contains a certain number of nerve fibres, quite constant from one individual to another, for example those which come from neurones in the fore part of the spinal cord in three main nerve trunks. If the number of nerve cells and fibres were somehow determined from the start, removing the normal limb or adding an extra or supernumerary one would not change it. Of course the final position of the fibres would be altered, but nothing else.

When the experiment is performed, however—and it is not
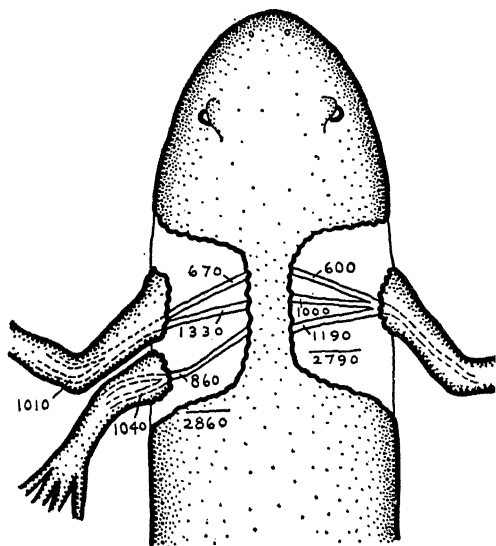
FIG. 151. A right leg was implanted on the left side of a salamander behind the normal left leg, and one of the three nerves was diverted from the normal to the added limb. After regeneration was complete, the number of nerve fibres was determined in each nerve branch. Though less than a third of the usual number of fibres was started into the added limb, the final number in it was the usual one that it would have had if left in its proper connections. (After Weiss.)

too difficult in an embryo salamander to amputate a limb bud or even to transplant an extra one—the results are clearly otherwise. In the animal with an amputated limb, the nerve cells which would have supplied it, and which have in no way been injured by the operation, largely fail to develop and few nerve fibres indeed appear in this shoulder region. Conversely, in an animal with an extra limb implanted just below the normal one, the small nerve which normally supplies the few muscles in this part of the chest wall becomes greatly enlarged and branched. The nerve fibres in it also divide so that finally there comes to be the same number of axones in the transplanted limb as in the normal one. Clearly the muscular requirements somehow help determine the nerve supply; but unfortunately this "somehow" remains completely unexplained.

In other cases, mechanical forces contribute to the control of cell numbers (and arrangement). The appearance of excessive bone cells at points of weakness in a fractured bone has been mentioned. In the same way, the stalk suspending a pear weighing four ounces may break under a load of six; but if additional weights are hung on day after day it will thicken and come to support a full pound. But even here the means by which stresses act remain unknown.

The related problem of how correct cell positions are assumed is also hardly beyond the stage of being clearly recognized. To return to nerve for a moment, we must ask not merely how the right number of fibres reach the muscles, but also how the nerves actually come to reach them at all; for normally the axones growing from nerve cells in fixed positions in the spinal cord "find" their own particular muscle. In the course of evolution some muscles have migrated far from their original locations to very different portions of the body, and their nerves have faithfully followed them. Yet, even in the adult, if the nerve normally supplying a particular bunch of muscles is destroyed in some way, fibres in nearby nerve trunks will sprout branches to take care of them, though ordinarily oblivious to these muscles.

If we examine another organ, the eye, this question of the proper position of cells becomes almost oppressive. Here is a high-grade optical instrument in which, as in any good camera, there must be placed in accurate alignment and at proper distances, shutter, lens system, other refractive media, and finally the photosensitive surface. In the camera, the various materials —metal, several kinds of glass, silver salt in gelatin emulsion— are separately manufactured from the most different natural sources and then assembled and mounted with precision machinery and human skill and intelligence. The eye, with its special transparent horny cells in cornea and lens, its shutter or iris of deeply pigmented and opaque yet soft movable cells, and its photosensitive retina of modified neurones and receptors, can perform feats of photography still beyond the scope of the most expensive candid camera.

Yet these highly varied cells all originate from the same amorphous blob of protoplasm, are all still, in fact, just one or another specialization of this life stuff; and, without help from some external guiding force or intelligence, for which there is no evidence, they have somehow managed to form and grow and move into exactly the right positions. It is small wonder that the biologist, when first taught by his dissections of the elaborate structure and machine-like perfection of function of the vertebrate eye, was stunned into a hopeless reverence for it. Such a marvelously efficient and adaptive instrument, he concluded, could only have been designed and built by a Godlike intelligence. But again mystery has yielded to analysis and it is now clear that the eye also forms under the control of material conditions, even though the mechanism of their action is still but partially understood.

Poets call the eye a window to the soul. Certainly it is a window to the nervous system, for the retina, its most essential portion, is formed directly from the brain and is really still a part of it. We must consider the earlier stages of development for an understanding of how the eye is built up.
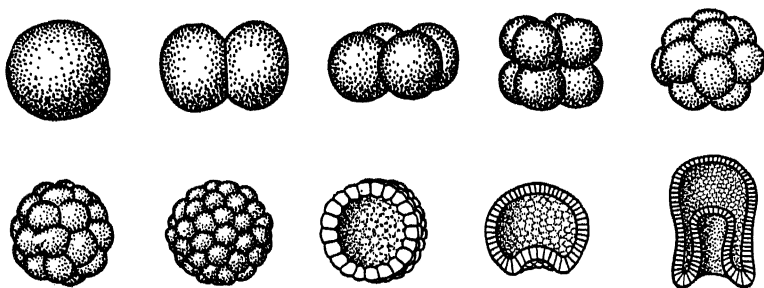
FIG. 152. The egg, after being fertilized, divides into smaller and smaller cells. These come to form a closed hollow ball (blastula) which invaginates to become a two-layered open hollow ball (gastrula).

After the egg is fertilized it rapidly undergoes cleavage in the three planes, one after the other, to give a solid ball of eight large cells. Continuing cell divisions produce a mass of smaller cells which soon come to occupy anything but a random position in a solid clump. The cells originally in the center of the mass push in among their fellows at the periphery and, after the first few divisions, all planes of cleavage are perpendicular to the surface. There arises, consequently, a hollow sphere composed of a single layer of cells which are packed in a close-fitting mosaic to make its wall, a blastula (little germ). From now on, cell growth between successive divisions is more prominent and the total mass of protoplasm increases in size, as well as in organization. The cells in one hemisphere develop more rapidly than do those in the other, so that the upper part of the ball tends to overgrow the portion below it. The result is an actual infolding of the less active regions, such as a child produces in a rubber ball by pressing in his thumb at one position. This gives an inner layer of cells which, as growth proceeds, becomes pressed close against the outer layer, so that there results again a hollow ball but with its wall composed of two cell layers instead of one, a gastrula (little stomach). Further, the cavity now is connected with the outside through the opening left at the point of infolding, the blastopore.

The main lines of the adult are already laid down at this stage, for the cavity becomes modified into the hollow inside of the digestive tract, the inner cell layer forms its walls, and the opening to the outside becomes, in various animal types, the mouth or the anus or even both. The outer cell layer becomes, of course, the skin and part of it, oddly enough, the nervous system. A third layer of cells, which forms later between the first two, is the progenitor of connective tissue, bone, heart and blood vessels, muscle, and many other structures. Soon after the double-layered ball is formed, the cells along one meridian, radiating from the blastopore, grow rapidly to form a thickened line. This marks the position in which the nervous system and backbone will develop and defines the main axis and middle plane of the adult-to-be.

The cells to either side of this line in the outer layer now grow very rapidly, and divide so that the new cells remain in the plane of the surface. As a result, this outer layer buckles into two folds, which parallel the midline. These folds of primitive skin increase in size as the cells still grow and divide, then lean toward each other and eventually meet, as a long arch over the midline. Then the outer layers of the two folds join across the middle to form a new continuous surface, and the inner layers similarly join and complete a hollow tube. This is the nervous system.

Fig. 153. From above down, stages in the formation of the hollow tubular nervous system by the folding of the outer cell layer of the embryo (gastrula).

So, by successive bursts of growth, migration, folding, and cutting off, one organ after another in the body is formed and properly shaped, much as a skilled glass blower manipulates his tubes under heat and pressure. Sometimes a whole sheet of cells migrates over others, partly under the growth pressure of

those behind. This can easily be shown by coloring certain cells in the living embryo with a non-injurious dye and then actually watching through the microscope as these stained citizens migrate in their cell society.

Now we can return to the eye, which first begins to develop long after these more basic parts of the body have been laid down. The neural tube has formed and the front end of it become partly ballooned out, to outline the future brain, when a region on each side of the front enlargement bursts into rapid growth. The old story is repeated and there results an extra
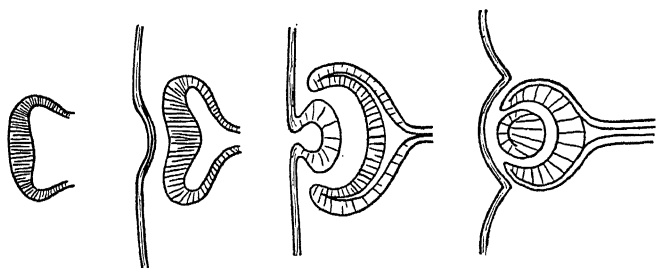
FIG. 154. Successive stages in the formation of a lens for the eye, from the embryo's skin, under the influence of an outgrowth of the brain—the optic cup—which will later form the retina.

bubble of cells, growing laterally from each side of the brain toward the skin. As these nerve cells approach the body surface they stimulate the cells of the skin to increased growth and so cause this to bulge inward. Finally a separate clump of skin cells is cut off, pushes against the neural ball, and invaginates it, much as happened earlier to the whole embryo. The main parts of the eye are now laid down; for the skin cells, after further growth and differentiation, become the transparent lens, and the nerve cup becomes the retina behind it. Of course many other parts have to be formed and oriented but they are produced by minor variations of the same motif.

Now, what of the important question, how do brain and skin manage to cooperate so perfectly in making their contributions

to the eye? Fortunately an answer is at hand. If the optic cup is cut away by microscopic dissection when it first grows out of the brain and is placed under the skin anywhere else in the embryo, say on the back, the skin above it is stimulated exactly as before and busily produces a lens. Such a developing eye, unconnected with the nervous system, is of course completely useless and indeed does not go on to full development. But the main point is clear: the skin growth coordinates with that of the brain because it is directly stimulated to do so by the presence of nerve cells near it. How this stimulus acts and in what way the responding skin cells are directed into the differentiation toward lens rather than epidermis, are other questions that are far from solved. But they are at the moment irrelevant to the main point; the machinery of development is geared together in a purely mechanical fashion, so that the completion of one step automatically initiates the proper succeeding one.

Chemical Control and Organizers

We can now face more directly the questions earlier left hanging: just what internal and external factors operate upon a cell lineage as it changes from undifferentiated potency to specialized efficiency; how do the various interrelations of parts and cells act to guide the individual unit and to integrate the whole organism?

No one has ever seen an oak tree patiently holding its roots on high while its trunk digs sturdily into the ground. Yet acorns contain young oak trees with tiny root, stem, and "leaves" already formed; and acorns fall upon the ground in all positions. As often as not, the root is pointing toward the sky and it starts to grow in this direction when the seed germinates. As soon as stem and root have extended beyond the open shell and are so freed in space, however, the stem turns up and the root down, even if they must bend through half a circle. How do these growing cells know "up" from "down" and what are

FIG. 155. The sunflower follows the sun from horizon to zenith and down again.

they directing their growth toward or away from? Many factors have been disentangled.

The stem, for example, always tends to grow away from the pull of gravity (negatively geotropic) and toward the source of light (positively phototropic). The root grows toward gravity's clutch (positively geotropic) and toward a source of water (positively hydrotropic). These tropisms are easily demonstrated in simple experiments which vary only one factor while keeping the others constant. So, for example, when light is directed upon a growing seedling from the side, instead of from above, the stem promptly grows more rapidly on the shaded side and so bends its tip to face toward the light. Everyone has watched the sunflower head similarly follow its sun god from horizon to zenith and down again. The relevant problem here is how the various stem cells are caused to elongate rapidly or slowly by the action of light.

New cells are formed by mitosis only at the very tip of the growing stem. Just behind the tip, these young cells carry on their stretching maneuver. Yet somehow the tip is still vital to this growth for, if it be amputated, the cells remaining, though apparently quite uninjured, do not elongate. A fascinating story

lies behind the further solution of this riddle, but we can look only at the last chapter. The tip cells produce a chemical, a growth hormone, which diffuses back along the stem and stimulates the elongation of the stem cells. The cut-off tip can be mashed and a cell-free extract obtained from it which contains the needed substances, for pouring this fluid on the truncated stem causes growth to resume. Or the cut-off tip can simply be placed on some porous solid, like gelatin or agar, long enough for the growth hormone to diffuse into the block, the latter then set on the cut stem, and growth again be produced.

A very simple experiment shows how light bends the stem. If the amputated tip is placed over the junction of two agar half-blocks and each is later tested on a separate truncated stem, the two will produce the same growth since each half had received
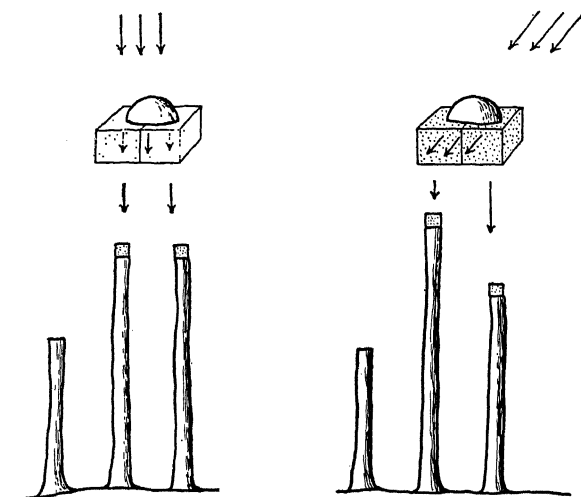
Fig. 156. In the experiment at the left, light came from above on the cut-off growth tip lying on agar. Each half of the agar then contained the same amount of growth substance, for the two halves led to equal growth when placed on the ends of seedlings whose tips had been cut off earlier. The third seedling (left), with no agar block and growth substance, remained short. On the right, the experiment is repeated with the light coming from one side. Tested as before, the results show that growth substance was "pushed" away by the light so that more was present in the shady half of the agar.

the same amount of growth hormone. But if the experiment is repeated while light shines on the isolated tip from one side, the results are not symmetrical. The agar block away from the light now produces a greater growth than before, that on the illuminated side a smaller one. The light acts as if it had pushed the growth substance in front of it so that, instead of diffusing straight down, it diffused down and toward darkness. In an intact stem, this process would, of course, insure a greater supply of growth stimulant to the shady side than to the sunny one, and the stem would always turn toward the light. Interestingly enough, the same substance that stimulates the growth of stem cells inhibits growth of those in the root; the shaded root cells are thus stunted and the root turns from rather than toward illumination.

Just such chemical agents, stimulating or inhibiting growth and favoring one or another kind of differentiation, are similarly at work in the developing embryo. These chemicals, or as they are called in this connection, organizers, stimulate not only cell division and growth but also differentiation and organization. They are formed most energetically by the cells in the lips of the blastopore, those which surround the primitive opening into the invaginated embryo. This is hardly surprising since it is this vicinity from which spreads the greatest developmental activity and in which starts each new burst of folding and thickening in the course of laying down the major patterns of the embryo. Working again under the microscope and with mechanically controlled hair-fine needles, bits of the embryo can be cut away and placed upon different
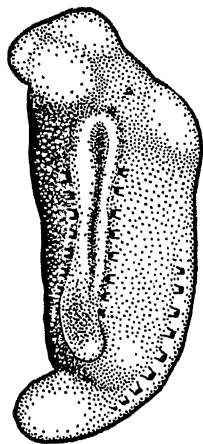
Fig. 157. A small second embryo has been caused to develop on the side of a normal salamander embryo by implanting in it a bit of "organizer." (Modified from Huxley and DeBeer.)

parts of other embryos. As with the growing plant, these bits, instead of being used as such, can be extracted and the organizers obtained from them applied to other embryos. Wherever these organizers are added increased development results, even to the extent of causing one part of the original embryo to sprout into an entire extra individual and so produce some sort of double monster.

Of course the organizers, for unquestionably many different ones are active together or in sequence, do not make all cells do the same thing. Most growing cells are further stimulated by them, but in which direction and to what final type depends largely on the cell stimulated and the phase of its development in which the stimulus is given. The amount or concentration of these substances is also important in determining their action. Organizers formed in a small group of cells in one place in the embryo will spread to neighboring cells by diffusion, as an ink-spot spreads on wet paper. With the cells placed in sheets, this will result in a disc-like spread and in progressively lower concentrations as one moves toward the periphery. The central manufacturing cells thus come to influence a halo of their fellows, the extent of determination diminishing with greater separation.

Such a "field" of control or action would slowly fade to zero at an indefinite distance were not other active centers producing their own fields in other parts of the embryo. These separate discs of influence meet one another at segments of their circumferences, so that some cells will come mainly under the influence of one field, other cells under another, until the complex mosaic of separate cell groups and patterns unfolds itself. Starting with just one most active region, which becomes the lip of the blastopore, the developing embryo acquires secondary, tertiary, and an indefinite series of other active centers, the interplay of which gives the final formed infant. The heart cells turning primitive gut cells into liver, or the brain cells in the optic cup which lead the skin to form a lens, are such sec-

ondary active centers controlling, probably by the diffusion of organizers, the cells within their field of influence.

Still later in development, when a circulatory system has been formed and the tiny blood currents sweep through the whole organism, substances elaborated at one point may control development anywhere in the body. By that stage development is far enough along to use the concepts and terminology applied to the ordinary adult or young individual, and such controlling substances clearly belong in the category of hormones. Of these, perhaps the most important are the hormones which control the developing cells of the genital system and help to determine a male or a female structure.

There is, then, in embryonic development a magnificent sequence and interplay of perfectly determined controls. Each cell with its changing potencies is subjected to environmental direction and regulation from neighboring and distant cells which, in turn, change their influence with their own development and that of the whole organism. There is here, indeed, an almost unrivaled display of the grandeur of life; for, through these eddying currents of change, movement, development, and shifting control, now one and now another unit or group accelerates its tempo or turns its direction, so that at the end the myriads of special cells are in proper organization to give a perfect and functioning normal individual—better than 999 times in 1,000. It is, in fact, a rare case in which connections have been missed, because of hereditary defect or developmental injury. These monsters are born, as a result, lacking limbs or eyes or, when the error is a late and minor one, with the upper lip still cleft, or the like; they serve to emphasize the amazing accuracy of the normal process of development.

Gradients and Individuality

It seems hardly possible that a single individual, organized as one total unit, could emerge from the interplay of the many

different and separated growth regions, and, indeed, there is part of the story still to be told; for over and above all the local fields and organizers there are other, still mechanistic, influences that act through the whole organism. These are, perhaps, the most interesting of all, and in some ways certainly the most important. They are also, unfortunately, less completely analyzed; for, though in some respects they act as if through the agency of spreading chemicals, this is often not the actual mechanism involved.

Let us start again with the unfertilized egg. If this is of the undifferentiated or indeterminate kind it is essentially homogeneous in all directions from the central nucleus. After fertilization a plane cuts it in two. Is the direction of this plane completely accidental or is it partly fixed, say by the point of entrance of the sperm, which point, itself, is determined by chance? In either case, the midplane of the embryo separates two identical and still essentially homogeneous cells. But now all further divisions are oriented with respect to this plane. The mitotic spindle must form in each cell, then, in a fixed orientation, since the plane across the spindle is that of cleavage and will pass accurately at right angles to the one already present. Somehow, the asymmetry in the cell—perhaps the difference in turgor or surface tension between the flat and the convex surfaces plus attendant changes in gelation and viscosity of the cytoplasm—does succeed in orienting the mitosis; but we must not stop to examine the large amount of study devoted to this problem.

The initial symmetry of the unfertilized egg, called spherical since the same conditions are met at the same distance along a radius in any plane, is temporarily disturbed during early cleavage but is again clearly manifest when the embryo has reached the hollow-sphere stage of development. At this time, the dozens of cells forming its curved wall are to all appearances equivalent. Yet at the next stage a permanent asymmetry is introduced, for then one part of the ball invaginates into

another and leaves a single open-
ing, the blastopore. The longi-
tudinal axis of the embryo
extends from the blastopore
through the center of the double-
walled sphere. No longer is
spherical symmetry present but
only radial symmetry, such that
a plane perpendicular to the
longitudinal axis contains like
radii diverging from the point
where axis and plane intersect.

It may seem academic to fuss
about the distinction between the
spherically symmetrical closed
ball and the radially symmet-
rical ball with an opening at
one end. Yet, in fact, the differ-
ence is one of the most pro-
found met in the whole course

FIG. 158. The blastula on the left
has the perfect symmetry of the
sphere—a plane in any direction
through a central point divides it
into like halves. This is no longer
true for the gastrula on the right,
which has the less perfect radial
symmetry of a cone or cylinder.
A plane, in any radius, passing
through the linear axis will divide
it into like halves, but any other
plane will not.

of biological evolution or animal embryology. The secret is
held in the last phrase of the sentence, "at one end." A sphere
and animals with true spherical symmetry have no end; but
practically all the more elaborated animals, which develop
through the infolded blastula, not merely have ends, but a
front end and a back end. Without some main body axis there
could be no head or tail, not even a stem with a leafy and a
rooty termination; but only ball-like organisms lacking a front
and back, a right and left, even an up and down, and possessing
only an inside and outside.

Here, in its simplest possible form, enters the general factor
which directs development of the whole organism. For it can
hardly be "chance" any longer that determines which part of
the hollow sphere is to grow faster and to fold in the remainder
and so to establish the front and back ends of the animal. It is

difficult to study the blastula of the sea urchin, because its cells possess cilia and the whole continually rolls about irregularly in the microscope field, but that of a hydra (related to a jelly-fish) is as easily obtained and normally stays on one spot. The fertilized egg is shed from the parent and slowly sinks to the submerged ground surface where it continues to cleave and develop until a blastula is formed. (Actually, this egg is discharged at a later stage than indicated and the blastopore forms in an anomalous manner. The discussion as to the place at which the blastopore appears is, however, valid.)

Note that this fertilized egg has spherical symmetry and is undifferentiated. No particular part of its circumference is more likely to land on the bottom, in contact with the ground, than is any other point. To make this absolutely certain, we can roll part way over some eggs that have landed and so leave a different bit of surface down. Now, where will the blastopore appear? If its position is predetermined by some unrecognized organization in the egg or by the point of entrance of the sperm or, the exact contrary, if its position is not determined at all but is located by pure chance, then the blastopore will have no relation whatever to the ground. The cells touching bottom, those opposite, or those anywhere else about the sphere would be equally likely to start the rapid growth that will finally invaginate those opposite to them. But this does not happen.

With machine-like regularity, the cells at the top grow faster than do those at the bottom and the hollow ball is folded in from below. The blastopore becomes the mouth and there is really little further development needed, merely the elongation of the double-walled ball into a double-walled cylinder, plus the sprouting of a few tentacles at the top end, to form the adult. The answer is, therefore, unambiguous. The point of formation of the blastopore is not set by any preexisting properties of the egg but is determined by the egg's position in relation to its substrate.

The further explanation of this behavior is still a long and

fascinating story. We might be tempted, after discovering the tropisms of plants, to see in this result another case of geotropism. That would be an error, however, for an egg on a highly slanted bottom still invaginates at the pole on the ground and no longer in the line of gravity. We can, of course, use another word, "thigmotropism" or responsiveness to contact, and say blastopore formation is positively thigmotropic. But this would be hardly better than a wordy cloak of ignorance; and actually contact is not the important control. There is another difference between the upper and under surfaces of the egg, besides the presence or absence of a solid contact.

The upper end is, so to speak, sticking its neck out into the world more than is its antipode. The cells on the bottom are highly protected from change and stimulation; above them is the relatively stagnant water in the cavity of the ball, below them the substratum, and on all sides the extended plate of their fellow cells. The top units are thrust by the height of the blastula, tiny though this be, into the watery vastness around them and so exposed to the feeble water currents that continually bring with them fresh food and oxygen and other exciting adventures. The bottom cells must receive these substances by diffusion from greater distances and often through other cells, the top ones bathe directly in their nutrient broth. Is it not reasonable that with more food and oxygen and greater stimulation they should be more precocious than their sheltered sisters?

This is not merely clever theorizing invented to explain the formation of the blastopore, but can be confirmed in several surprisingly different ways. The top cells remain in a more exposed position throughout life and, if exposure really does lead to an increase in their activity and metabolism, this should be directly demonstrable. It is. Direct measurements of the rate of various metabolic processes in different parts of the animal show that a piece of the top or oral end uses more oxygen, contains more food substance, and grows more rapidly

than a piece of equal size from the basal region. In fact, as body
regions along the wall of the animal from bottom to top are
examined, there is found a steady increase in metabolic rate;
an intensity gradient with a spatial extension.

It would be possible to continue the analysis in these lowly
coelenterates, but everything is so much more striking, almost
diagrammatic, in a more evolved group of animals, the flat-
worms. These gliding bits of narrow ribbon, perhaps an inch
long, have made the final jump forward in the plan of animal
organization. Planaria is no longer sessile, as is the sea anem-
one, but moves about through its environment. Like its more
primitive relatives, it has a differentiated top and bottom; al-
though, since it captures its food mainly from the surface over
which it slides, it has turned over and keeps the more active
mouth end down. But it has had to solve also the problem of
front and back, or head and tail, which, of course, carries with
it the related one of right and left.

A radial animal has no longitudinal organization and there-
fore has no forward or backward direction of movement. It
tends to stay put and, if stimulated, merely pulls in its "horns"
and collapses into as small a volume as possible. Even if it
were inclined to run from danger it might find itself going in
several directions at once; and, in-
deed, the radial starfish with its five
walking arms has been seen, when
suddenly stimulated, to do just this
—a couple of the arms start pulling
toward the deeper water, the others
toward the shore, and in the result-
ing tug-of-war the animal may ac-
tually tear itself in two. (This isn't
as serious as it sounds, for each
fragment proceeds to grow the miss-
ing arms and two healthy starfish
result. New England fishermen,
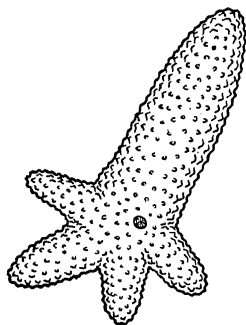
FIG. 159. One arm of a star-
fish regenerates the missing
arms.

bothered by these animals fouling their nets, tried to eliminate them by cutting them up and throwing the parts back into the ocean. Their war of annihilation was, not surprisingly, a dismal failure.) This is the reverse solution from that of the storied ass, who starved to death between two equidistant and equally alluring piles of hay because of inability to decide which to enjoy first. Both types of behavior are equally unsatisfactory.

Let us ignore the time element of sluggish evolutionary change and dramatize, in a single animal, the shift from radial coelenterate to polarized flatworm. Suppose water currents flowed predominantly from one direction, so that a constant part of the animal's circumference were chronically exposed to better food and oxygen supply, more changes in temperature, the gentle rain of chemical and physical stimuli. Applying again the same arguments used for the developing blastula, this stimulated surface should become more active metabolically and somehow take the lead. If the animal were then forced to move, this active end would get under way earlier and pull harder, and so set a direction. But such a process must build up like an avalanche. If the animal moves with the most active cells kept in front, then even more than before these front cells are likely to encounter new adventure more effectively than those which follow behind.

The result should be an animal with established front and back ends, legalized anatomically by structural differences, which we call head and tail. The body plan is altered in that the line of the vertical axis, around which the original ancestor had been radially symmetrical, becomes stretched into a fore and aft plane. All the more evolved animals are bilaterally symmetrical; that is, the halves produced by a cut from head to tail vertically and in the midline are essentially symmetrical mirror images.

According to this interpretation, there is a remarkable reciprocal relationship between structure and function. The head end gets the bumps and the bumps make the head end. What

evidence is there for such a surprising conclusion as this? First of all, even more clearly than in the coelenterates, metabolic gradients are demonstrable in the flatworm. The oral-aboral one, which is still present, and another, which diminishes from the midplane laterally, need not concern us. For, dominating both of these is a much stronger one from front to back. This can be shown in the same way as before. Equal-sized bits of flatworm, taken at similar intervals of distance from head to tail, show a progressively and regularly diminishing rate of respiration and of allied chemical activities. There is no question that the level of cell activity grades off smoothly from the highest intensity at the head to the lowest in the tail region; the metabolic gradient is there all right. But how is this related to the differentiation of particular cells and organs so that the head structures are always at the high end and others at the appropriate level along the line?

The mere existence of such a gradient is itself impressive. It means that, as the cells are actually lined up next to one another, say in the body wall, each one is a bit more active than its tailward neighbor and a little less so than its headward one. Suppose a thousand men gathered in a field were suddenly told to form a line from north to south. Would it not be rather amazing if the line were found to have the tallest man at the north end and every individual standing in the order of his height to the undersized fellow at the south? It would, in fact, be so utterly unlikely that, when such a human file is seen, we are quite certain that the men have been deliberately lined up on the basis of stature. Yet such an explanation of the similar lining up of cells in a sequence paralleling their initial degree of activity is quite impossible. These units do not migrate around to any such extent, and there is no marching of the more vigorous ones to the head of the class. We are forced to just the opposite conclusion. Instead of the more active cells going forward, the forward cells must become more active. The bumps make the head. The placid country lad, thrown into
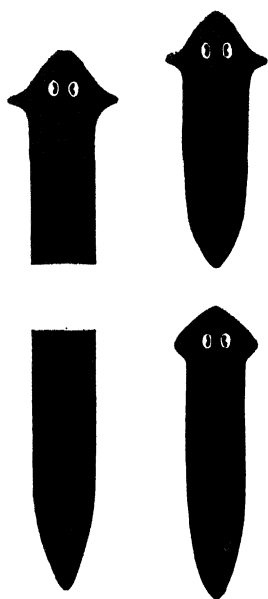
FIG. 160. When a flatworm is cut in two, the back end of the head piece gradually changes into tail and the front end of the tail piece into head. (After Child.)

the more stimulating human environment of the city, lives at a more accelerated tempo than does his brother left on the farm.

Happily, all this can actually be demonstrated on the flatworm. An animal cut across the middle survives and each half regenerates the missing parts. The cut end of the back half rounds into a blunt headless stump from which the normal tail runs backward, and the front piece becomes a tailless head. Soon, however, the foremost cells in the tail stump begin to grow and differentiate. They become reorganized into a head region, from which the missing parts of the body later develop by backward growth. Measurements of metabolic rate taken at these stages show that the head-forming cells have greatly increased their respiration and, in fact, only after this increase has occurred does the structural differentiation into a head proceed. The cells at the back of the original head piece show no signs of becoming a head and no increase in metabolism, but they do grow backward to form a new tail whose cells show the low respiration proper to a tail region.

The crucial point is this: some particular cell that happened to find itself just behind the initial cut greatly increased its metabolism and became heady; but had the cut gone a fraction of a millimeter farther back this same cell would have found itself at the back end of the front piece, its metabolism would have remained moderate, and its descendants have become tail. Clearly there was nothing in the cell itself to determine which

of these very different paths it would follow but only its position relative to the other cells. If it were the most active of the lot it, so to speak, took over responsibility and reorganized itself and its subordinates, otherwise not.

There is no question that the heightened activity is responsible for the structural change into head, for if some cells in the tail are deliberately irritated by various stimuli, such as the continued application of chemicals, their metabolism is increased above that of neighboring cells and, even though no cuts are made, these active cells proceed to form a new head. The worm thus becomes a tandem individual but it soon pulls apart to form two perfectly good worms.

But how does this increase of metabolism come about in the cells behind a cut? Those at the end are, of course, somewhat stimulated by the operation itself, but this is equally true for cells on both sides of the wound and only those of the tail piece rise to new metabolic heights. Of course, the front cells on the tail piece are now the ones directly buffeted by the environment, the ones that get the bumps, and this is surely one factor that brings about their increased activity. But another more important force is at work, that of organismic integration which we have been seeking. The mere taking away of the more active cells in front of it somehow releases the cell behind.

All cells are normally kept subordinated by more active cells which dominate them; except, of course, the foremost head cells, which rule the roost. How this relation of dominance and subordination is effected we do not quite know. In some cases, when a nervous system is present, it can be shown that the more active head end continually sends inhibiting or subordinating nerve messages tailward; in other cases, mechanical forces enable the oral end to dominate by acting faster and more powerfully, as in normal intestinal peristalsis; in still others there is perhaps a backward diffusion of inhibiting substances, much as in the case of the growth-inhibiting substances seen to control prototropism in roots. It is possible, by

cooling and other means, to stop the passage of such impulses or substances or to block some other kind of mechanism of control without actually cutting apart the plant or animal, and then a new head begins to develop (or in the plant a new growing tip) somewhere down the line.

If such a quantitative organization, with dominance and subordination of parts, seems at first a bit mysterious it can be made extremely familiar by considering an army. Regard each man as a cell, the army the organism. It has a head, not identified especially in space (though generals are rarely in the front line), but in importance and control. The general dominates his staff officers, they control ones of lesser rank, and so down through the military hierarchy to the subordinate private—a continuous gradient of importance, power, and responsibility. The functions of an individual are determined by his level in the gradient. In any one unit, when the senior officer is lost, his immediate subordinate assumes command and becomes the new head. He actually increases his military stature and, if kept in this position, takes over completely the official structure and function of his former chief. This is closely analogous to the cells at the front end of the amputated tail. With their dominant colleagues removed they are no longer held in check but take over fully the responsibility and privileges of control, with a concomitant freedom to increase their own metabolic stature. It is rather less likely that all privates would be lost while the officers remained; but if it did happen, the most junior officer would have to descend to the role of private just as the cells at the back of the headpiece become tail.
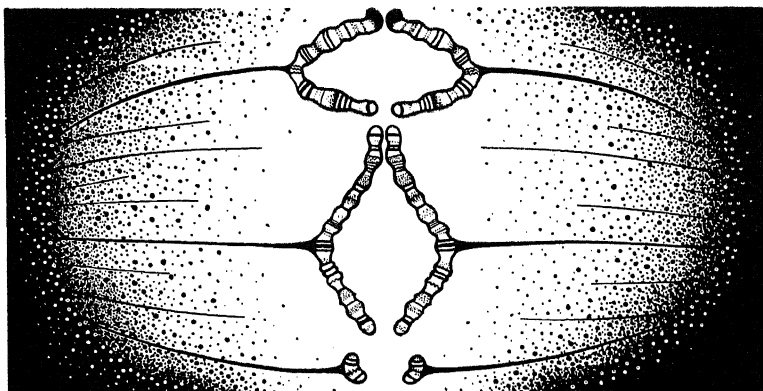
Chapter Thirteen: Inherited Molecules—
Heredity and Genes

Hans Andersen told a fairy tale over which adults the world over, no less than children, have smiled and wept ever since. It is the story of the ugly duckling hatched from the same brood of eggs as did his more standard fellows, and in the same barnyard. The infants were coddled and prodded alike by their conscientious mother, but the ugly duckling never fitted in. He was born different and he remained different; and Andersen wrote a delicate sociological treatise upon his progressive persecution and final ostracism by the group. Of course the story ended well, with the metamorphosis of the bedraggled little creature into a lustrous swan and the pointing of a final moral: "It does not matter in the least having been born in a duckyard, if only you come out of a swan's egg!" That sentence is perhaps as good an epitome of genetic knowledge as has been made, and this chapter will be concerned in expanding it.

We have seen illustrated time and again that each kind of cell possesses an individuality of function and of structure, which is based ultimately on a specific composition and especially on its proteins. In individual development, the offspring of the toti-potent egg become progressively specialized. Apparently, on

a much more majestic time scale, the same thing has happened to the eggs themselves; all eggs have a common ancestry in the earliest life forms, yet each egg can now produce only its own kind of organism. What, then, have the parent swans contributed to the swan egg which insures that, if it become anything at all other than, perhaps, part of a salad, it will be another swan?

It is certainly one of the major triumphs of biology that this question can be answered in great detail; and oddly enough the answer has come twice, from two sets of quite unrelated investigations. The classical story is now all but hackneyed and few have failed to hear of the Austrian abbot, Gregor Mendel, who bred tall with short and rough with smooth sweet peas and followed the occurrence of these physical characters through generations of their offspring. From breeding experiments, of course with intact animals, continued over the long-time intervals required to study many generations, it has been possible, nay necessary, to draw conclusions as to the happenings of ultramicroscopic bodies within the nuclei of single cells during seconds or minutes. For heredity is a problem of the chromosomes and the substances they carry, plus the vagaries, or perhaps better the certainties, of chance in dealing them out.

Breeding Guinea-pig Fur

The domestic guinea pig can exhibit a remarkable range of color, pattern, and texture in its hair. Individuals continually appear, in various colonies, ranging from pure white through yellows, browns, and reds to pure black; from uniform color to spotted, mottled, streaked, and mixed, with all the possible colors splattered about as if by the mischievous brush of a Walt Disney. But any one hair is always just a single color. A close relative of this domestic pet is the wild Peruvian cavy, probably the stock from which the guinea pig was derived, and a more distant one is the Brazilian cavy. These small rodents
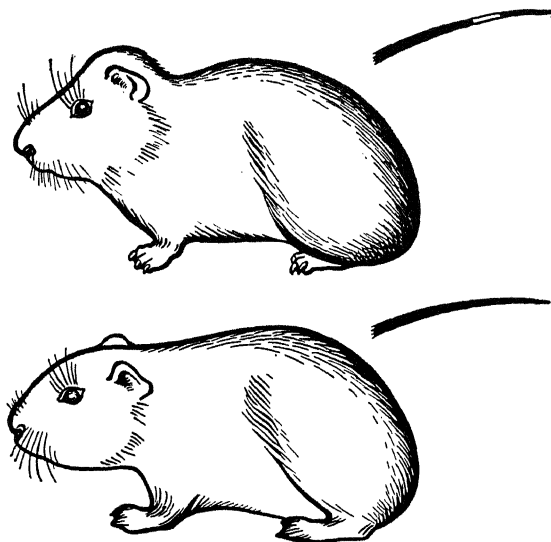
Fɪɢ. 161. The cavy, above, resembles but is distinct from its relative the guinea pig, below. Its hair, a neutral gray brown, has a band of ˌyellow near the tip, giving the "agouti" pattern. Though guinea-pig hair may be of almost any color, it does not normally have any banding, but each shaft is one solid color.

lack the chromatic display of their relative and face the world in a desirably inconspicuous fur shaded a dirty gray-brown, technically called agouti. The color is unique in that each hair has a band of yellow near its tip. The dark agouti color pattern of the Brazilian cavy is never seen in the guinea pig.

Now, although these two animals are unmistakably different, and in many other characters besides hair color, they are still so nearly alike in their proteins that it is sometimes possible to cross the female of one species with the male of the other and obtain healthy offspring. It doesn't matter whether the father or mother is the agouti or, for the main results, whether the guinea pig parent is black or yellow, of a flat color or spotted. All the children resulting from the mating have the agouti color, although in respect to the other physical traits some may be distinctly guinea pig and some distinctly cavy. Obviously the

cavy parent, male or female, has contributed something which completely dominates any contribution of the guinea pig so far as hair color is concerned.

To find out more about the "something," individual children of this hybrid generation can be mated with other cavies, or with guinea pigs. Mating with the cavy produces grandchildren of little further interest, for no matter how often this is done the offspring are all agouti; and their further descendants, on being crossed over and over again with wild cavies, continue indefinitely to show only the agouti color. But the cross of the hybrid and guinea pig produces a fascinating litter; half of these new babies are agouti, like one parent and the cavy-grandparent, the other half are as clearly guinea pig in color. Of course, in any one litter this 50 per cent inheritance is not likely to be exact, but as thousands upon thousands of similar families are followed, the proportion of agouti to, say, black becomes ever more accurate at just one to one, or 50 per cent of each.

The existence of this ratio is of great importance, but of greater significance now is a conspicuous non-existence. Every individual grandchild is either completely agouti or completely black. There is not the least sign of a mixture or blend with some spots of agouti and others of black, or some hairs of an intermediate shade, or even one single hair of the wrong kind relative to its fellows. This agouti-producing "something" is either completely there or completely missing; there are no halfway measures. This makes the whole matter seem more precise and tangible, for only definite unit material things are present or absent in such uncompromising oneness.

Yet perhaps we are being too enthusiastic in drawing this conclusion and are dealing, after all, with a situation more like that of pouring liquid into a dish on one side of a balance. A critical amount will tip the scales, less is ineffective and much more may be present without additional effect. The grand-parent cavy may have supplied so much of the dominant agouti

agent that, even though it has been diluted over two genera-
tions, about half of the grandchildren still have enough to turn
the trick. Note that it would be an unlikely chance, were this
the case, that exactly half should pass the agouti threshold, but
it is not impossible.

The question can easily be settled by further breeding
studies. The original offspring had one-half cavy inheritance
and one-half guinea pig. These 50 per cent cavy animals were
mated with 100 per cent guinea pigs, black of course, so that
the second generation, receiving a heredity of one-half pure
guinea pig and one-half 50 per cent guinea pig, is 75 per cent
guinea pig. Or, put another way, each time a cavy hybrid is
mated with a pure guinea pig, the amount of cavy in the off-
spring is only one-half of that present in the cavy parent.
Surely, then, if we continue breeding the mixed stock with pure
guinea pigs we can dilute this hypothetical cavy fluid and
obtain descendants which, in successive generations, are in
ever-larger proportion colored like guinea pigs; until finally the
agouti is completely lost. If a bottle of ink were stirred with an
equal amount of water, the original container then filled with
the mixture and this again stirred with a like volume of water,
and so on, the color would progressively fade and finally be
quite undetectable.

But the animals do not behave this way. As might be ex-
pected, the 50 per cent of grandchildren already colored black,
when mated with black guinea pigs, give only black offspring;
these third-generation animals again mated with black guinea
pigs, give black offspring; and so on. The interesting case, of
course, is breeding agouti grandchildren with black guinea
pigs. The result of this third-generation mating is exactly like
that of the second, 50 per cent of the issue are completely
agouti, 50 per cent black. Yet these great-grandchildren are
only one-eighth cavy, whereas their grandparent generation
was one-half cavy. This test can be repeated generation after
generation, and every time an agouti is mated with a black
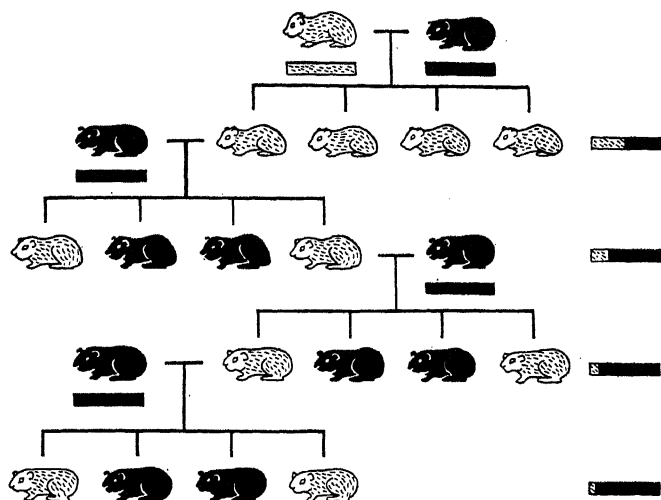
Fig. 162. The original mating of a cavy (stippled) with a guinea pig (black) gives offspring which all show the cavy hair, though they are only "50 per cent cavies." Further breeding of such cavy-haired animals with black guinea pigs always produces cavy hair in half of the offspring (on the average). This is still true in the fourth generation of children, though by now the cavy-haired animal is almost "95 per cent guinea pig."

some biological machine accurately grinds out half black and half agouti in the descendants. Such experiments have been continued rigorously for nine generations so that the final offspring possessed, compared to the original cavy parent, only one-half times one-half times one-half nine times, or less than one five-hundredth of the original agouti agent; and more routine breeding has continued until the agouti was diluted millions of times.

Clearly our early conclusion is completely vindicated. The original cavy contributed some definite material entity to the stock and each individual descendant from then on either got it in toto or missed it entirely. The question next promptly arises, how could one such unit from one individual animal go to hundreds or thousands of offspring and each still receive a complete unit? One answer would be, of course, that this heredi-

tary unit, like an individual cell or organism, grows and reproduces itself; but we may have some qualms in applying such biological concepts to the multiplication of particular chemical substances. Let us leave this for the moment and agree that the experiments from breeding prove that there is a unit transmitted from parent to offspring, which confers upon the individual possessing it agouti-colored hair, and which can multiply as do the animals in which it is lodged.

We had fixed our attention on hair color in these breeding experiments and therefore selected for mating, in each generation, those individuals possessing the agouti shade. Other physical traits did not run parallel with hair pigment, so that there was no directed selection in regard to these. Sooner or later, for every other character, the situation became that which would have occurred for color if the black instead of the agouti offspring had been chosen. That is, for every attribute but color, the hybrid parent chosen happened sooner or later to be one which completely lacked instead of completely possessed the cavy type. As a result, every other cavy characteristic was lost along the way and these ninth-generation offspring are completely normal guinea pigs in every respect but one; they are colored agouti.

We have, then, evidence for the conclusion that the hair color of a guinea pig is dependent on some material unit which was present in the egg or the sperm which fused to produce the animal. Such a unit was incorporated in the germ cell during its formation in the parent, but only when it had been likewise present in the egg or sperm which formed that parent. The unit is not, however, passed on as a single entity, like some valuable family heirloom such as a silver urn. A non-growing inheritance would either progressively be subdivided among generations of children until diluted away, as often happens to a fortune of money; or would go only to one descendant in each generation to the exclusion of the others, as the entailed titles in England pass to the eldest son. Biological inheritance does

not behave in either manner. The inherited unit, like a living tree but not like an inanimate piece of silver, itself grows and multiplies. If, on establishing a new family household, husband or wife brings along a cutting of a prized tree long in the family, then the offspring of this union might in turn carry with them fresh cuttings for their own families. If neither parent brought such an inheritance, of course no child could receive it.

But a self-multiplying inheritance might or might not be received in its entirety by each child. In our analogy, for example, social custom might demand that cuttings of the family tree be taken only by sons. This recalls the case of inherited hair color in guinea pigs; for, when one parent brought the agouti inheritance into the family, not all but approximately half of the children received it. The problem then must be faced, what biological machinery of selection or shuffling parallels, in the organism, the action of social custom in the parable of the tree? But before answering this, largely with the aid of the microscope, let us take a final look at the breeding experiments.

Units of Generation

We have deliberately followed the presence or absence of agouti hair color and have related it to the presence or absence of a discrete inherited unit. A number of things have been discovered about this unit and it is proper to give it a label, if for no better reason, because we must refer to it constantly. It has come to be called the gene, a nickname for "unit of generation." The presence, then, of a particular gene, let us say agouti gene, in the fertilized egg which will form a particular guinea pig, leads to the appearance of agouti-colored hair in the developed individual. But what of the guinea pig with black hair or yellow or spots of both? It would seem probable that similar particular genes, identified as black gene, etc., are involved in these tints no less than the others, and indeed the same sort of story has been worked out for them.

If hair color is determined by a certain gene or genes, why not similarly eye color, hair length, leg length, leg structure, eye structure, head form, and in short, each of the innumerable details of anatomy which collectively constitute a guinea pig? Why not look upon the fertilized egg as a sort of mail-order catalogue containing one latent unit of almost everything, one gene for every characteristic of the adult, which, each playing its individual role, collectively produce the bundle of traits known as an adult? Early in this century, when genetic triumphs were pouring out of the laboratories, many biologists held just such a simple picture of gene action before themselves. Further investigation has, as so regularly happens, analyzed the simple into a new complex. Most genes influence one another's actions and an elaborate interplay between them is the rule, with few exceptions. Not one but many genes contribute to an adult character; an agouti or a black gene is only one of many which interact to produce the final color. And, conversely, each gene plays its part in molding a whole group of adult structures. Further, there is a continuous cooperation between the inherited units and the environment in which they unfold; else some of the findings in this chapter would be almost irreconcilable with those noted in the preceding one.

One important type of interaction has already appeared. The offspring of agouti and black parents receive, through egg and sperm respectively, both color genes, yet these animals never develop into mixed agouti and black. The very first cross made between cavy and guinea pig, we recall, produced only pure agouti litters. The black gene was, none the less, present in these babies, not only by necessity if our picture is right, but also by the actual demonstration of further breeding; for these agouti hybrids, when mated with pure-white guinea pigs, will produce some offspring which are just as black as the original grandparent. All genes, then, are not of equal "potency" but certain ones may take precedence over or dominate others.

The agouti gene is dominant over the black one in the sense that, when both are present together, the former expresses itself fully and entirely suppresses the action of the latter.

A similar relation can easily be shown between pure-black and pure-white animals and is, in this case, perhaps more intuitively obvious. Suppose an albino is an animal which has simply failed to inherit an essential gene for color. The descendants of a mating between an albino animal and a pigmented one, black or another tint, would then come to express whatever color genes they inherited rather than remain white. But the same breeding results would follow if albinos possessed a gene for white which is recessive to all other color genes. Any other gene, then, would be dominant to white and manifest only its color when present. The cases of black domination over white and of agouti dominating black would then be strictly equivalent, though different sets of genes are concerned in the two cases.

As another example of gene interplay, consider pure-white guinea pigs that have come from a long line of albino ancestors. Any matings between animals of this particular white strain produce only white offspring. In some other laboratory, another inbred strain of guinea pigs has likewise produced, for many generations, only white animals. If white indicates the absence of other color genes, then, clearly, the mating of animals from these two different stocks could result only in additional albino descendants. When the experiment is performed, this usually proves to be the case; but sometimes the offspring are colored. Actually this discovery was made on breeding white sweet peas rather than guinea pigs, but the same inexorable logic should be applicable.

The investigator who first crossed two separate stocks of white sweet peas and obtained some offspring with lovely purple flowers was much disturbed by this factual heresy. Certainly, if one gene produces color and no other gene influences it, two white plants could not parent a colored family. Since

this is just what happened, the original premises about gene action could not have been entirely correct. He devised a more complex hypothesis; two different genes acting cooperatively are required to produce color, either one alone is ineffective and white results. Any particular white strain may lack color-gene-one or color-gene-two or both, but it would be impossible a priori to tell which. If, however, in the course of albino matings, one chanced to breed an individual possessing color-gene-one with one of another strain possessing color-gene-two, then at least some of the offspring would possess both color genes and actually be purple.

The disturbing case in sweet peas is thus accounted for; but the hypothesis is not proved by its working in the situation for which it was designed. Actually, however, as applied to a vast number of characteristics of many plants and animals, such a hypothesis has been shown to account for not merely the qualitative kinds of offspring produced but also the quantitative proportions of each kind. Some further elaboration has been needed. About a dozen different kinds of genes, for example, are known to contribute to the production of hair color in guinea pigs, and there is every reason to suspect that additional ones will be uncovered as experiments continue. Such cooperative genes are appropriately called factors, thus implying that no simple cause-and-effect relationship exists between each gene and some body characteristic, but rather that many genetic factors operate to bring about the final result.

The case just considered is one of complementary factors, the action of either gene complementing that of the other. The converse relation is also well known, in which the presence of one gene, an inhibitory factor, prevents the action of another; something like the depression of black by agouti. In other cases, one factor may supplement the action of another by modifying its manner of expression. A supplementary gene, for example, is needed to change red into purple; without it the sweet peas would have turned out red. Still others show

cumulative action, a piling up of like effects, so that either of two factors alone may suffice to produce a light pink while the two together give a darker red of the same hue. An amazing amount of information has already accumulated about particular genes and their actions. Roughly a hundred have been identified in man and about 500 pairs in the intensively studied fruit fly. Our present interest, however, is not in genes as bricks in a remarkable monument to scientific exploration but rather as biological units with amazing powers of action, of which a clearer picture is desired.

To a chemist, certainly, the existence of such things as complementary factors would be no surprise but the very thing to be anticipated. If a gene is a particular substance, obviously not itself red or black or agouti colored, it must lead to the appearance of these colors in the adult hair by causing red or black or brown pigments to be formed. But one substance, of itself, rarely turns into something else; the great bulk of chemical change depends on an interaction of several substances. Water and carbonic acid (with the proper enzyme and energy) give sugar and oxygen; either one alone does nothing. So also many genes interact with each other and other substances to produce the final materials and structures in the body. We might, even at this stage, think of one factor as supplying substrate and another as enzyme which catalyzes its changes. Before pursuing this problem, however, it is necessary to return for some further direct evidence of the character and behavior of genes as a class.

Chromosome Cotillion

During mitosis, the ordinary process of division of body cells, changes within the nucleus dominate the picture. The original clear globule becomes hazy and a fine chromatin strand appears in it, and this thickens and shortens until it finally breaks up into fragments, the chromosomes. The most striking fact in
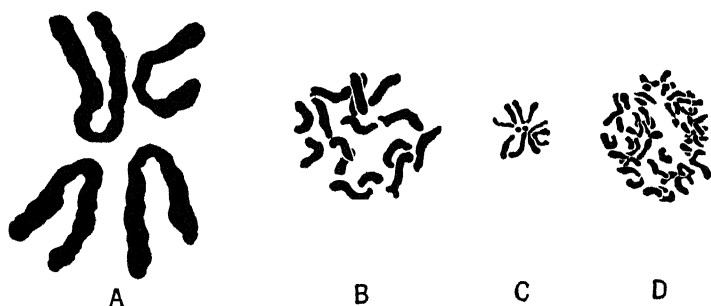
Fig. 163. Actual appearance of chromosomes during mitosis, each group being characteristic of one species. A, round worm; B, pea; C, fruit fly; D, man. (From several sources.)

all the already amazing, Lilliputian maneuvering is this: the chromosomes of every cell in all individuals of any particular species of plant or animal appear in a size, shape, and number characteristic of that species and of no other. If we knew nothing else relating these nuclear elements to heredity, this alone would be a broad hint that some essential connection between them does exist.

Another important fact, indicating that the nucleus plays a predominant role in transmitting the array of hereditary characters from one generation to the next, has to do with the egg and the sperm. The egg from the female parent brings to the union a small nucleus and a relatively large amount of cytoplasm, while the sperm from the male parent contributes a small nucleus (the head of the sperm) only. Yet the sort of breeding experiments we have just been examining show, with great consistency, that the male and female parents contribute equally to the offspring. It makes no difference whether the male of one pure breeding line is crossed with a female of another, or the female of the first line with the male of the other; the subsequent history through all generations of the family will be the same. It follows, then, that the nucleus, contributed alike by both parents, is responsible for transmission of indi-

vidual traits while the much larger amount of cytoplasm, coming only from the mother, is inactive in this respect.

Further examination of the chromosomes under the microscope brings out another relationship. The number of chromosomes varies widely in the cells of different species, between two and two hundred (over a thousand in some plants) but, in normal individuals, this number is always even. Further, the chromosomes occur in pairs; that is, a chromosome of one particular size and shape will always have another like one associated with it. (To avoid an important misstatement, it is necessary to mention that one and only one chromosome pair is made of individuals which may differ from each other, which may even entirely lack one of its members. These are the so-called sex chromosomes, and the presence of two unlike ones largely determines one sex, the presence of two like ones, the other. This fascinating chapter of heredity fits beautifully with other knowledge but must be left untold since it adds nothing to the understanding of genes, which is the problem before us.)

In the course of mitosis, the full array of chromosomes becomes centered in the plane midway between the points of the spindle, that plane along which the parent cell will ultimately split into its two daughters, and then each individual chromosome neatly splits in two (really duplicates itself) lengthwise. In man, with forty-eight chromosomes, this maneuver results in the production of two sets of forty-eight daughter chromosomes. One complete set later moves in each direction away from the center plane, and finally becomes incorporated in one of the descendant cells. This machinery clearly guarantees the accurate apportionment of chromosome material, in amount and kind, to each cell. It would seem to insure a regular reduplication of the original cell type.

But what of the first step of all, the fusion of egg and sperm? Each contributes a nucleus and its chromosomes and, if the routine mitosis were run through without special adjustment, the number of chromosomes in the species would double with
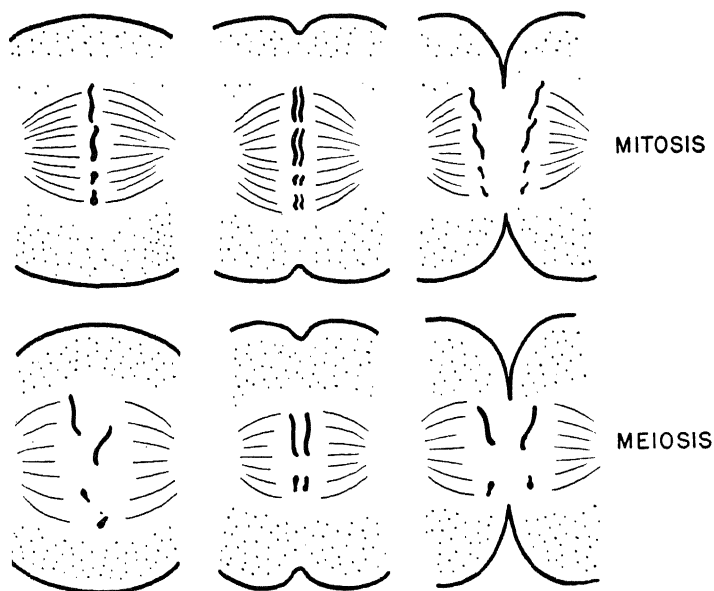
Fɪɢ. 164. In mitosis, each chromosome of each pair divides into two. In meiosis, the two chromosomes of each pair separate from each other with no division.

each generation. As might be expected, this dilemma has been solved in a very neat fashion; the manner of solution, in fact, gives the clue to the whole problem of hereditary units. Both eggs and sperms originate from particular parent cells in the adult animal which are descended, through a series of cells formed by orthodox mitosis, from the original fertilized egg. In the final stages in the production of gametes, however, these parent cells divide to form eggs or sperms, as the case may be, in a different manner. The chromosomes no longer line up individually in the center plane but move up to it in pairs and, instead of forty-eight single chromosomes all splitting, the members of the twenty-four pairs merely separate. Each of the final germ cells thus comes to contain but one member of each pair. This special reduction division, or meiosis, gives each gamete

just half the original chromosome number; and when two unite in the act of fertilization, the full complement is automatically reestablished. Note, further, that one member of each of the twenty-four pairs of chromosomes in every cell of your body was contributed by your father, the other by your mother.

Now let us look at the next generation, to be formed again by the fusion of an egg and a sperm. The egg parent cell possesses both paternal and maternal members of each chromosome pair; the egg will contain only one representative of each. Which chromosome will be lost to it? The possibilities are few and obvious. All the chromosomes originally received from the father, or the mother, might be retained and the alternate ones relinquished; or the paternal element might be kept in certain determined pairs, the maternal element in others; or the whole thing might be entirely a matter of chance, either component of any particular pair having the same likelihood of being retained or lost. These three possibilities would lead to very different kinds of inheritance, providing that the units of heredity, the genes, are contained in the chromosomes, and that certain particular ones are in each. Let us assume for the moment that this proviso holds, and go back to our guinea pigs.

On chromosome C, let us say, of the original cavy ancestor was a gene that tended to produce agouti-colored hair. On the equivalent chromosome C' of the black guinea pig ancestor was the gene for black. These particular chromosomes were present, respectively, in each egg and sperm and were, therefore, both incorporated during mitosis into every cell of all the offspring. These individuals, we saw, were all agouti colored, because of the action of the dominant agouti gene. When the sperms of a son and the eggs of a daughter were in turn being formed, only one chromosome of this pair could enter each. Half the sperms, therefore, would contain chromosome C with its agouti gene; the other half, chromosome C' with its black gene. The eggs would similarly be divided in like number between those with the agouti gene and those with the black.
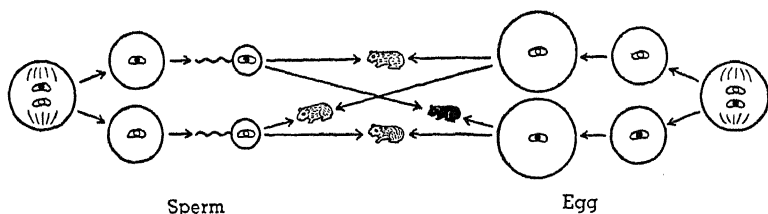
Fig. 165.   As a result of meiosis, each sperm (left) and each egg (right) has one gene of each gene pair. Two kinds of sperm and of eggs are formed in equal numbers, and either sperm may fertilize either egg. If one or both sex cells carries a cavy gene (∞), the guinea pig produced will have cavy hair; only if the cavy gene is entirely lacking will the animal be black.

If, now, any sperm is equally likely to fertilize any egg, four kinds of grandchildren would be produced in equal numbers as follows: C-agouti (from sperm) C-agouti (from egg); C-agouti (from sperm) C'-black (from egg); C'-black (from sperm) C-agouti (from egg); and C'-black (from sperm) C'-black (from egg). Actually, as we have seen, it makes not a particle of difference in the behavior of any gene or chromosome whether it came from the mother or father, the egg or sperm, so that two of these types will be identical. All the offspring produced by inbreeding the children of the original cross will thus fall into three genetic groups, of which one will occur twice as often as either of the others. The grandchildren will be: 25 per cent C-agouti C-agouti; 50 per cent C-agouti C'-black; and 25 per cent C'-black C'-black. Of course those individuals possessing two agouti genes will show this color. Further, those with one agouti and one black gene will likewise appear as agouti, as did their similarly constituted parents. Only one-fourth of the offspring, the 25 per cent that chanced to get black from each parent, will actually show up with black hair.

The only way to distinguish the individuals with a double dose of agouti from those with a single dose is by further breeding. On being mated with a black female, let us say, which produces eggs with only the black gene, the two agouti males

will produce different color ratios in the offspring. Since the sperms of the father with a pair of agouti genes must each receive one, all egg and sperm unions will give the same black and agouti gene combination and lead to agouti children. The father with agouti and black genes will produce, in equal numbers, sperms containing each one; half the fertilized eggs will have two black genes; and 50 per cent of the offspring will turn out black, 50 per cent agouti.

These results with hair color explain fully the sharp differences that occur between children of the same parents; but what of the evidence as to how different chromosomes are apportioned to an individual germ cell during meiosis? For this it is necessary to follow the action of two different chromosomes and note whether a particular member of one pair always appears with a particular member of another, as it must if any fixed type of segregation is involved; or whether either chromosome of one pair has an equal chance of appearing with either of the other pair, as it must if the dealing out is strictly random. This is very easily done.

Suppose an agouti pig with smooth hair is mated with a black one with rough hair. Smooth and rough, exactly like agouti and black, have been shown to depend on a gene pair and the answer is given by the behavior of these two separate characteristics. The sperm will contribute a C-agouti chromosome and an R-rough one, the egg a C'-black and an R'-smooth, and the resultant offspring will contain the alternate individuals of each pair. When it, in turn, becomes sexually mature and produces germ cells, only one member of each pair will be included in each egg or sperm. If the distribution is predetermined, either the C and the R chromosomes will always appear together (and, of course, the C' and R'), or the C and R' (and the equivalent C' and R). This will be the case for both eggs and sperms. If the first alternative were correct, all offspring would have one of the following combinations: CR CR; CR C'R'; or C'R' C'R'.
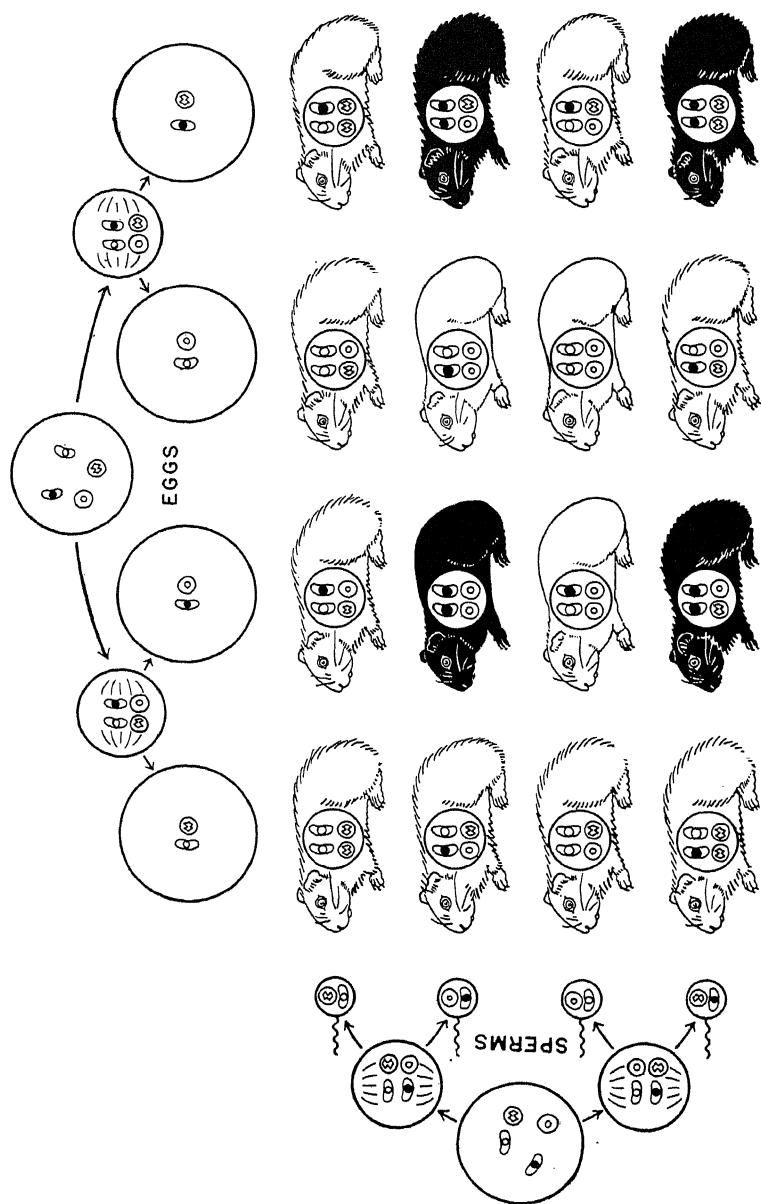
There could be no case in which, for example, the combination CR′ CR′ appeared. Conversely, if meiosis had produced CR′ and C′R germ cells, no case of CR CR could appear.

But if each of the two pairs of chromosomes segregates independently of the other, then four kinds of sperms, and of eggs, would be produced instead of two. There would be CR, CR′, C′R, and C′R′ sperms and the same varieties of eggs; and, since any sort of sperm could fertilize any sort of egg, four times four or sixteen combinations might result. Actually, several of these turn out to be identical and the final kinds of offspring and their proportions work out as follows:

1 CR CR	2 CR C′R	1 C′R C′R
2 CR CR′	4 CR C′R′	2 C′R C′R′
1 CR′ CR′	2 CR′ C′R′	1 C′R′ C′R′

All animals with one or two C chromosomes, carrying the agouti gene, will show agouti color, but only those with the double C chromosome will continue to breed true. Similarly, those with the R chromosomes, carrying the rough gene, will have rough hair but only those with two will pass it on to all offspring. Even without these breeding tests, however, it is obvious from the above genetic formulae that agouti and roughness will be quite independently inherited. On crossing a pure agouti rough (CR CR) and a pure black smooth (C′R′ C′R′),

FIG. 166. The ancestral cell, about to form eggs or sperm, may line up two pairs of chromosomes during meiosis in either of two ways. Each way produces two kinds of gametes. Four kinds of eggs, and of sperms, will therefore be formed in equal numbers. Any kind of sperm may fertilize any kind of egg, giving the sixteen combinations shown. Only the kind, not the source, of the genes in the offspring is important, however, so that many of the combinations of genes turn out to be identical. Even more, many animals with different gene combinations—which would breed differently from each other—still look alike. When either one or two doses of the dominant gene for rough (⊚) is present, the animal's coat is rough; only when both recessives (◎) are present is the coat smooth. Similarly, one or two cavy genes (⬯) lead to cavy coloring and only when both recessives (⬮) are present is the coat black. The important point here is that the genes in each chromosome pair are dealt out by meiosis quite independently of the other—so all possible combinations occur in the descendants.

there should result a pure-breeding agouti smooth (CR' CR') and a pure-breeding black rough (C'R C'R), as well as the original two pure-breeding strains and every possible mixture. This is exactly what happens. Clearly the different chromosome pairs are sorted into the germ cells in complete independence of one another. It is now apparent why, in the original experiment on mating a cavy and a guinea pig, it was possible, by selecting agouti color but neglecting all other cavy characteristics for many generations, finally to obtain animals whose characteristics were all pure guinea pig except for the agouti color.

Strings of Beads

Another problem is now confronting us. The fruit fly has only four chromosome pairs, yet over five hundred genes (or factors) have been identified in this insect. If genes are on chromosomes then surely several hundred must be carried by each. But if this is true then all those genes on a particular chromosome should be sorted out together in meiosis and not independently. If, for example, hair color and hair texture were determined by genes on the same chromosome, the independent shuffling of these characters could not occur.

Suppose agouti and rough genes were both on chromosome A and black and smooth genes on chromosome A'. The germ cells would get either one of these chromosomes, always with its fixed pair of genes. Fertilization would give three kinds of offspring—with chromosomes AA, AA', or A'A'—but there would always be an equal number of agouti and rough genes linked together, and likewise an equal number of black and smooth. The new fashions in guinea-pig fur, agouti-smooth and black-rough, would not appear. In fact, if all the genes on one chromosome were irrevocably linked together in this manner, it would be very difficult to identify them as separate individuals; for ten or a hundred which collaborate to produce certain traits would regularly be present or absent together and

so would act like a single one. Yet just such linkage of genes has been demonstrated and, with one important loophole, it works in just this way.

Had our picture of genes and chromosomes been as sharply etched a third of a century ago as it is now, it would have been predicted with great confidence that many genes must be on one chromosome and would necessarily behave in this linked fashion. Actually, the empirical discovery of this phenomenon of linkage came first and gave tremendous additional support and impetus to the establishment of these interpretations. Not merely does linkage exist, but it has been possible from a careful study of its workings to decide which genes occur together on one chromosome, and which on another.

In the fruit fly, two of the chromosome pairs are made of long slightly angulated individuals, a third pair of short stubby rodlike ones, and the fourth of small spherical dots. If we allow imagination play and make a mechanistic picture of how genes are arranged in a chromosome, the simple one arises of the genes, like beads, strung together in longer or shorter chains which occupy the available length of the chromosome. Of course such a crude model could hardly be expected to reflect accurately the true situation. The more surprising, then, that when the genes are allocated by linkage measurements it turns out that they occur in four groups with the following partial number in each: 12, 100, 111, 144 or in the following ratios: 1:8:9:12. The ratios of length of the four kinds of chromosomes are 1:7:10:12. Possibly the naïve picture of a string of beads is not so naïve after all.

Well and good, we shall continue to think in models. Can one go further and say, not merely which beads are in a particular string, but in what order they are strung? Possibly we are past being surprised, yet it is an amazing tribute to the accuracy of analysis in this field that the answer is an emphatic "Yes." The evidence comes from still another trick in chromosome behavior.

Fɪɢ. 167. When chromosome pairs come together during meiosis the two indi-viduals are commonly much tangled together. Sometimes, when they again separate, parts are exchanged. Drawings of three such pairs as seen in dividing cells. (After Morgan.)

In meiosis, the two chromosomes of each pair line up side by side in the midplane before they wander apart into the two daughter cells. As seen under the microscope at this stage, they ordinarily appear not stretched out next to each other but rather somewhat tangled together or partially twisted about one another. Does it seem unreasonable that now and then, when the two come to separate, instead of neatly slipping apart they might actually tear in two and the back half of one become at-tached to the front half of the other? Of course, beads on a thread would not behave so unless somebody carefully re-knotted the torn ends after the string was broken; now surely this is carrying the model too far. But remember that in the resting nucleus the individual beads, if they are lined up in strings, are not in a chromosome jacket but hold together by some attractive force.

Well, reasonable or not, let us suppose that portions of the chromosomes of a pair can be accurately exchanged for one another, so that the group of genes originally on one crosses over to the other. Let us assume further, that crossing over

FIG. 168. Microphotograph of two cells in a root tip, at early and late stages of mitosis. Magnification over 1000 times.

occurs only rarely but with a certain fixed numerical probability, say one chance in every ten instances of meiosis. Finally, since everything else about gene distribution has turned out to be a matter of chance, let us assume that the point along the chromosome at which the two partners exchange portions may also be located at random. What will be the consequences?

Think of the letters A to Z arranged in alphabetical order on chromosome one and the equivalent set, A' to Z', on chromosome two. We can calculate how often meiosis will end up
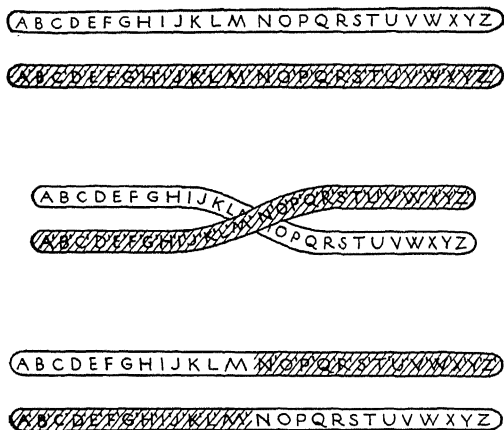
Fig. 169. A pair of chromosomes with the many pairs of equivalent genes. Crossing over is illustrated.

with a chromosome the front end of which still has gene A but the back end of which, by crossing over, has gene Z' instead of Z. Since an exchange anywhere along the chromosomes would separate the original A and Z and since an exchange some-where occurs one time in ten, the AZ' combination will result in every tenth instance. If we consider the gene N instead of Z, then a chromosome exchange anywhere in the first half will combine N' with A but none in the second half will do so. The chance of this happening, if the genes are equally spaced, will be one in twenty instead of one in ten. By the same reasoning, a chromosome containing genes A and B' will result only from an exchange between A and B and not from crossing at any other possible position, so this should occur only one time in 250.

If, then, a sufficient number of breeding experiments were performed to identify various genes on a chromosome and to find out how often the different ones of a pair were inter-changed, one could reason backward and obtain their positions relative to one another. Thus, in the fruit fly, if genes for eye color, wing shape, and bristle length are found to be all

normally linked together, and therefore presumably on one chromosome, the following experiment can be performed. A pure-breeding fly with red eyes, long wings, and straight bristles is crossed with another pure-breeding fly with white eyes, miniature wings, and forked bristles. The offspring will obtain from one parent a chromosome with one set of three genes (for red, long, and straight), and from the other parent the paired chromosome with the three equivalent genes (for white, miniature, and forked). When their germ cells are being formed by meiosis, one or the other chromosome with its original three genes will be included and the respective three characters will be present together.

Except for crossing over. Actually, in tens of thousands of such cases, it turns out that 54 per cent have red eyes, long wings, and straight hairs; but red eye, miniature wing, and forked hair come out together in 26 per cent; and red eye, long
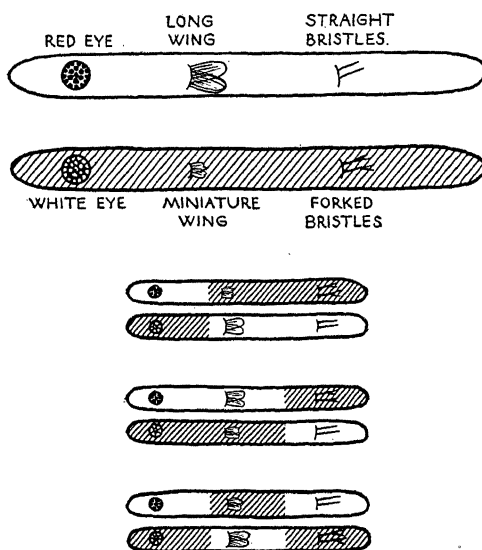
FIG. 170. Three gene pairs on chromosomes of the fruit fly. From the percentages of crossing over, shown by the results of breeding, the positions of the genes can be determined.

wing, and forked hair in 15 per cent. Red eye, miniature wing, and straight hair appear together in 5 per cent, because of a double crossing at both ends of the chromosome. Clearly, the gene determining wing shape lies between that for eye color and that for bristle form, and crossing over may leave it with either of the others. An unbelievable amount of careful study of this kind has succeeded in assigning to practically all the genes in the fruit fly a position and order on the various chromosomes. Even more, from relative crossing-over frequencies, the actual linear distances between each one and its neighbors have been calculated. Chromosome maps have been drawn, with the position of every gene, identified and named in terms of the characters it influences in the adult individual, located with as much precision as are the houses along the map of a country road.

Surely, this time, scientists have let their mental pictures run away with their better judgment and are spinning extravagant theories from a few poor shreds of evidence. This was more or less true until a few years ago, and even the investigators making these exact quantitative tests and drawing their neat maps and conclusions were careful to point out that they should not be taken too literally. Yet an entirely new and unexpected line of evidence has not merely justified these pictures in detail but actually made them visible through the microscope.

The ordinary cell nucleus is some ten to twenty micra in diameter and a single long chromosome within it is perhaps ten micra long and one-tenth this distance across. A micron is toward the far end of the range of resolution under the microscope; and, although an occasional investigator, using magnifications of 3,000 diameters, claimed to distinguish the finer structure within a chromosome, most workers mistrusted the findings obtained by pushing a technique to the very limit of its possibilities, or perhaps beyond. It seemed, therefore, hopeless to expect direct visual evidence of the genes in chromo-
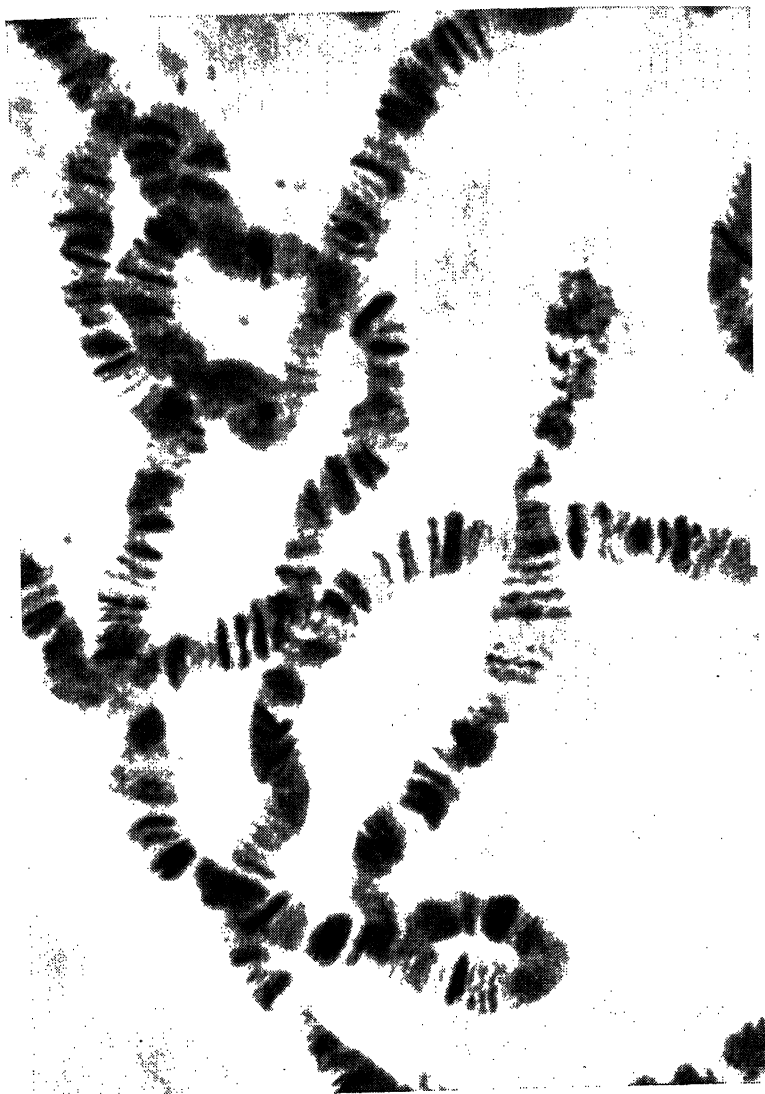
FIG. 171. Microphotograph of giant chromosomes in a fruit fly cell, magnified almost 3000 times.
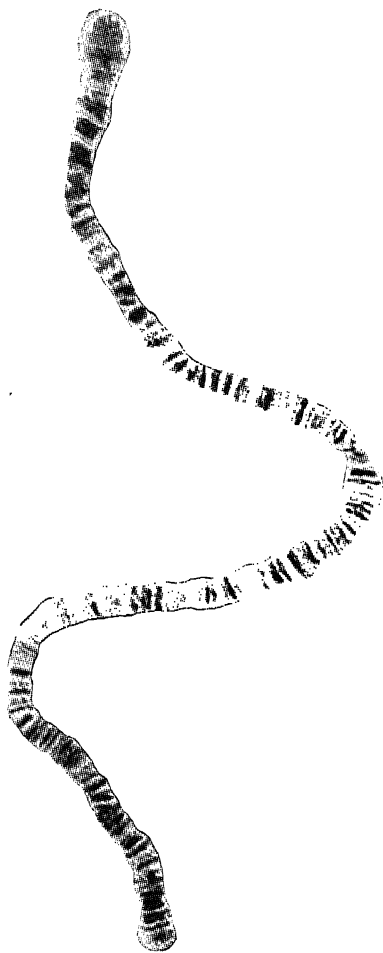
FIG. 172. Microphotograph of a single giant chromosome. (After Painter. From *The Journal of Heredity*.)

somes. And then the accidental discovery was made that, in the salivary gland of the fruit fly larva, there exist relatively enormous cells which contain giant chromosomes during mitosis. These have the same general characteristics as ordinary chromosomes but are over a hundred times longer and much fatter, built from them, presumably, as a cable is from like wires.

Such giant chromosomes are easily seen under low magnification, can actually be discerned with the unaided eye, and their finer structure can be read with the microscope as clearly as the print on a page. The simple thread previously visible becomes a long rope, with characteristic swellings appearing at regular positions and with cross striations forming wide and narrow bands of different transparency down its length. The effect is that of a long series of discs of varying diameter and thickness piled upon one another like a stack of dishes. The number of discs that is visually distinguishable, about six thousand, corresponds to the number of genes previously estimated to be on the chromo-
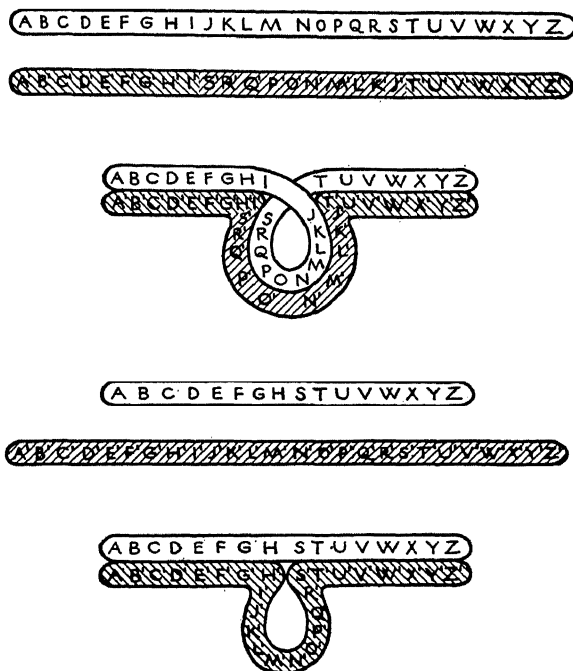
FIG. 173. Above, the middle portion of the shaded chromosome, carrying genes J to S, has become reversed. In meiosis, its mate forms a loop so that the genes in each chromosome will be in proper pairs, J to J and S to S. Below, another type of abnormality in which part of the unshaded chromosome has been lost. The shaded mate then forms a hump so that those genes present in both still match.

some, and the thickness of the discs parallels the gene separation on the chromosome map.

In further details also these giant chromosomes have added their bit. Apparently the alternate genes of some particular pairs, such as G and G′, do not look exactly alike, and the corresponding disc has one appearance in a chromosome known by breeding to contain G and a slightly different look in a chromosome known to contain G′. The exchange of chromosome parts has been directly observed and even some highly abnormal cases have been noted. Instead of a proper crossing

over between two chromosomes, an extra bit of the second may become attached to an otherwise unchanged one, or an exchanged bit may hook on wrong end to. In these exceptional instances the offspring that result are abnormal and in exactly those respects that should follow the known gene irregularities. Further, in the pairing of chromosomes, equivalent genes on each seem to attract one another so as to lie neatly side by side. When a piece of one has become reversed so that the genes read, say, AB; GFEDC; HI, etc., then the second chromosome forms a loop in itself and thus brings its genes to match those in its abnormal mate. A more satisfying confirmation of scientific theory could hardly be desired.

A Set of Chemical Reagents

The evidence so far, then, seems to lead irrevocably to the position that certain material units, genes, (1) possess such power of multiplication that each can divide to produce two identical copies of itself, (2) are present in specified positions on identified chromosomes in cell nuclei, and (3) are able to act, singly and in cooperation with each other and the environment, to produce in the cell and organism that possess them the innumerable single attributes which collectively constitute that particular individual.

Does such a list of properties give any clue as to just what a gene is? A definite entity in a definite place certainly indicates a particular chemical substance. The highly specific character of individual genes is reminiscent of proteins in general, and their ability to reproduce themselves, a type of autocatalysis, also suggests enzymes and so proteins. Further, the action of minute genes in the cell and organism, to bring about the formation in quantity of many new substances and structures different from themselves, while they remain at the end in their original form, again is strongly suggestive of the behavior of enzymes. Finally, on a more philosophical level, the problem

of transmitting from one generation to another the enormously complex attributes that characterize any particular species could hardly be solved in a more elegant and economical fashion than by the handing on of a relatively small collection of autocatalytic enzymes. These are able to produce, first, more of themselves; second, other active catalysts; and, only later, by the action of these enzymes upon the raw non-specific materials plentifully available in any sort of foodstuff, the specific structural materials of the new body.

A chemist traveling to a far and primitive land, to develop there a complete laboratory, would have to take various items of equipment (that is, a certain minimal organization) and a carefully selected set of chemical reagents, so chosen that with their aid he could manufacture from the natural resources of the land all the substances he would need. Pioneers everywhere, spores from the mother civilization who must travel light, do not carry the objects in use in that civilization—planks, hewn logs, bricks, bullets, lye; but rather the tools with which to produce them—saws, axes, molds, kettles—and tools to produce the tools.

All inferences, then, point strongly to the protein and enzymatic nature of genes; what more tangible evidence supports or refutes such a guess? Of course, the most direct evidence would be some sort of chemical test, but this is obviously not so easy to obtain. A partial step in this direction was taken in the last century by analyzing spermatozoa. Since a mass of these is composed overwhelmingly of nuclear material, including chromatin, there is at least some chance that any substances present uniquely or in specially great concentrations in this material do represent the chromosomes. Such special materials are, indeed, found and they are sufficiently different from the ordinary proteins to merit the special name, nucleoproteins. Disappointingly, though, they are among the simplest of the proteins known rather than, as would have been anticipated, the most complex. These are combined with another group of

substances, the nucleic acids, also reasonably specific for nuclear materials. But, of course, this is hardly proof that any of these substances may properly be identified with chromosomes, still less with the genes themselves.

A more direct approach has only recently been successful. Microchemical tests for proteins have been applied to dividing cells and, under the microscope, the chromosomes actually seen to give the proper protein reaction. This establishes part of the case fairly decisively but still does not prove that the genes themselves are protein. Perhaps they are quite different sorts of molecules embedded in a protein matrix to form the complete chromosome. This possibility cannot be directly resolved until the gene itself can be seen, identified, and tested; perhaps this will be possible with the giant salivary gland chromosomes.

Some further evidence comes from a consideration of actual sizes. A single protein molecule, as we have seen, may vary in molecular weight from a few thousand to as many million. If arranged in globular form, when the mass of a single molecule varies a thousandfold the diameter changes by a factor of ten. The known proteins fall in the range of 10^{-7} to 10^{-5} cm. The diameter of a single slender chromosome may be considerably less than 10^{-4} cm., so that there is some likelihood that the whole structure is no more than a single linear strand of protein molecules lying adjacent to one another. It has, in fact, been calculated from the number of known genes present in one sperm head of measured volume that the average diameter of a single gene must be less than twice 10^{-6} cm., probably much less.

So much for the evidence, admittedly not yet conclusive, that genes are proteins; what of their role as enzymes? The story here is even more fragmentary, but a considerable number of suggestive facts have come to light and others are rapidly being discovered. Two examples will indicate their nature.

One true-breeding strain of corn has all the kernels in all ears

closely packed with masses of discrete starch grains. Another equally constant strain has kernels which are not starchy but more homogeneous and waxy, and chemical tests of them show the absence of starch and the presence of simpler carbohydrates and fats. The sperm of the corn, as of all flowering plants, is present in the tiny pollen grains, which contain little else. Pollens collected from the two kinds of corn are found to differ in two important chemical aspects. That from the starchy corn contains a little starch and practically no enzyme which hydrolyzes it. The pollen from waxy corn, on the contrary, contains no starch but does contain considerable amounts of the enzyme, amylase, which splits up any starch to which it has access. It seems that the waxy corn possesses a gene, absent in the starchy variety, which is either itself a starch-destroying enzyme or else leads to the production of such an enzyme. The differences in the mature plants would then be a fairly direct expression of the presence or absence of this catalytic gene. When a hybrid individual is produced by crossing waxy and starchy parents and its pollen grains are tested chemically under the microscope, it turns out that half of them have the usual starch content and half have none. This is exactly what should result when meiosis has separated the chromosome pair, carrying respectively the waxy and the starchy genes, and dealt one or the other to each germ cell.

The second example comes again from hair color, this time studied in the rabbit as well as in the guinea pig. The pigment which makes black hair black, called melanin, is produced by the oxidation and combination of tyrosine molecules. Tyrosine is one of the regular amino acid constituents of proteins and, therefore, is amply present in the food and the protoplasm of white rabbits no less than black. It is, however, a fairly stable substance and changes to melanin only when acted upon by a special enzyme, tyrosinase, able to juggle this molecule and apparently no other. The color difference would easily be understandable if the cells of the albino rabbit lack the enzyme,

while those of the black possess generous quantities of it. This can readily be shown to be the case, for a piece of skin from the black animal yields on extraction an active tyrosinase solution, skin from the albino, none; or, even more, the proper substrate added to skin cell slices forms large amounts of melanin in the one, none in the other.

Clearly, one factor that is inherited in the black rabbit and not in the white is the enzyme tyrosinase, or some agent for manufacturing it. It has not yet been possible to test the germ cells directly, as in the case of corn, but at least this additional reason exists for ascribing the presence of tyrosinase to a particular gene; it is inherited, and it behaves in various breeding crosses exactly as does a single hereditary unit. Further, the color of the final animal does depend very completely upon the germ cells from which it is formed rather than upon the color of the parent in which it develops. The neat experiment was performed of transplanting the ovary from a black rabbit into an albino in place of its own. Sometime later, the white mother was mated with a normal white. The offspring of the union, apparently of two white animals, were consistently and completely black; the white mother in which the embryos developed had no influence at all on the black heredity.

Metamorphoses of Molecules

Another basis for assigning catalytic properties to the genes is found in the great body of data concerning mutation. This phenomenon, originally discovered as a natural process, has more recently been induced experimentally. Mutations are sharp changes in the activity of individual genes, suddenly acquired and permanently retained. At least, the following findings seem to demand such an interpretation.

A particular strain of plant, self-fertilized through tens of generations, has bred thousands of white-flowered individuals with never a suggestion of color. Then, though the routine

breeding is continued just as before, there appears on one single plant a solitary red flower. The eggs in the white flowers, when fertilized by their own pollen or that of other white flowers, continue as before to produce pure white plants. But the red flower, allowed to pollenate itself, produces seeds which grow into new plants in the ratio of three with all the flowers red to one with all white. Two of these reds on self-fertilization again produce three reds to one white; but the third one becomes the ancestor of a pure-breeding red-flowered race which, except in flower color, remains identical with the original white. Further, when one of these pure reds is crossed with a white, all immediate offspring are red; and when these are inbred one-quarter of the second generation are again white.

In other words, the descendants of the original red flower possess a gene for redness which may be present in single or double dose; which is the companion or alternate to the original gene for white, which is self-perpetuating; and which behaves throughout long breeding experiments exactly as does any long-established respectable member of the gene community. Yet we know it to be a parvenu, for prior to the appearance of the single red flower this red gene was just as clearly absent as it was clearly present from then on. One of the original pair of white genes in some ancestral cell somehow suddenly changed to a new individual gene able to produce the red color. From this cell were formed by mitosis all the cells of this one blossom, including the all-important germ cells as well as those of the petals.

Such mutations are continually occurring and, in a thoroughly studied population like the fruit fly, a certain probability has been found for different particular genes mutating. A gene contributing to the formation of red eyes mutates once in every three hundred cases to one giving only white; whereas a gene necessary for developing normal wings mutates to one yielding miniature wings only one time in ten thousand.

The stories of evolution, of the origin of species, and of the

segregation of racial groups are intimately bound up with the existence of mutations; for the appearance of a new kind of a plant or animal reduces, in the last analysis, essentially to the origin of novel genes which confer the specific characteristics on the new individual. Mutation continually introduces new gene performers into the continuous vaudeville of existence, there to show their tricks and to be judged by their works. As the traits they originate are of service or disservice to the animals possessing them, these organisms will flourish or fail; produce many successful offspring or few inadequate ones. And the gene will prosper or perish with the organism that bears it.

Natural selection inexorably operates over sufficient time, but on genes even more certainly than on individuals. The injurious genes, those which induce non-adaptive or retrogressive changes in the organism, soon disappear from the race. A mutant red caterpillar survived in the laboratory as well as the original green ones, but in an open roof garden the red ones soon disappeared as bird food. The neutral genes, producing a characteristic of neither greater nor lesser survival value than those which they replaced, will survive without being particularly favored. If individuals possessing them should chance to become isolated from the original stock, a new species may come into existence; one different from, but neither more nor less successful than, the original stock. Finally, the genes which introduce new or altered characteristics which give their possessor an advantage—greater speed, stronger jaws, more or sturdier young, immunity to infection, greater intelligence, etc. —will be selectively favored. Individuals possessing them will constitute a larger fraction of the population of each succeeding generation until the mutant gene has painlessly but completely replaced the one from which it sprang.

Now what does the evidence from mutation have to do with genes as catalytic molecules? First of all, the abrupt and considerable change in action of a gene is just what would be ex-

pected if some specific molecule underwent a chemical reaction to become a similar but definitely different substance. There would be no gradual shifting from white to red, but a single quantal jump. Let us be unjustifiably specific and say that some particular molecule leads to whiteness, while the same molecule with two hydrogen atoms oxidized leads to redness. To see how possible such a situation is, we need only recall the inactivity of oxidized glutathione and the high catalytic potency of its reduced relative; or the similar difference in hydrogen atoms between male and female sex hormones.

Secondly, a particular gene may mutate in one or very few cells of an organism while its identical fellows in adjacent cells, even its companion in the same one, remain entirely unaffected. This is shown in the case of the white plant with the single red flower. (Incidentally, certain genes in somatic cells mutate with great regularity. This has been proven for color patches on some flowers; and perhaps the white spots on a black guinea pig are a consequence of the mutation of black genes to white ones in those cells of the embryonic skin from which the white patches are descended. Since the presence of color spots is itself inherited, and breeds in the usual manner, this is a case in which the presence of a particular gene in the germ cell manifests its action by causing other genes to mutate in the petal cells which are formed later in the development of the individual. An inherited gene that acts by causing mutations in other inherited genes—this is indeed piling Ossa on Pelion.) Such localized change is entirely understandable if the gene is a single molecule which is partly responsible for the chemical manufacture in its own cell. Whatever it is that leads to the alteration of the molecule of one cell may leave untouched an identical molecule in the neighboring one. But of this more shortly.

In the third place, the wide range of frequencies of mutation for different genes again speaks for a chemical change in the molecule. It is the most elementary chemistry that certain

kinds of molecules are more reactive than others; that hydro-carbons, like kerosene, are oxidized with great difficulty, while their oxidation products, alcohols, are relatively easily oxidized; that silver chloride is decomposed by light while sodium chloride is not touched. If gene mutations are alterations in particular molecules, then they should occur more often in the chemically reactive ones than in those which are more stable.

A fourth point which is obvious, but no less important, we have touched on before. The mutated gene does not itself possess the new organismic attribute but only engenders it. In the white-flowered plant, the new gene produced red petals but not red sperms or eggs. Even in the red cells of the petals, the nucleus and its chromosomes remain colorless; the pigment is all out in the cytoplasm. The gene, before or after mutation, is not red, though in one state it cannot and in the other it can induce the formation of the red molecules. But clearly this is a change in the catalytic activity of the gene molecule, its own chemical composition being important only as it determines this activity.

A fifth point is worth mentioning here, although its full implications have not been presented. The genes of any particular species are certainly a highly aristocratic committee of chemicals. These are the ones selected for survival in the course of evolution; the ones that have passed the fine sieve of selection in terms of the useful characteristics they induce in the adult body. Out of the billions of molecules which are possible, these few hundred or thousand have been carefully passed down from generation to generation as a biological heritage. Then a mutation occurs in one and it becomes a different molecule. What are the probabilities that the effect of the new molecule in the organism will be better than, worse than, or equivalent to the original one? What are the probabilities that a monkey wrench dropped at random into a printing press will improve or disrupt its performance? If our picture of genes is correct a like answer must hold in both cases; the overwhelm-

ing number of mutations should be inimical or disastrous to the organism in which they occur. This is the case. Of many thousand gene mutations which have been observed under laboratory conditions, not one has led to an improvement in the plant or animal possessing it, though occasionally it has favored survival under altered temperature or other environmental conditions. A few have produced indifferent changes, as in color pattern; many have led to disabling deformity, such as dwarfed wings, color-blind eyes, or absent thyroid; and a great number have been directly lethal, the organisms in which they occurred being so deficient that they could not even survive until birth.

Finally comes the evidence from experimentally induced mutations. What sort of agent could be responsible for provoking sudden chemical alterations in particular genes of particular cells while leaving unaffected other cells or different kinds of genes in the same cell, or even the second gene of a single pair? A great number of chemical, physical, and biological agents have been experimentally directed against cells without affecting to the slightest degree the rate of spontaneous mutation. One reason for this is obvious; if any considerable damage were done to a cell, or any considerable number of its genes were changed, that cell would die at once or after a few abnormal divisions and without producing any organism at all. Indeed, a great many of the attempts to produce mutation led to complete sterility in the animals exposed or to the production of embryo monstrosities.

The problem was finally solved by the use of X-rays in carefully tempered dosage. A single X-radiation may pass through or between billions of molecules without being itself deflected and without disturbing them. Then it happens to strike one atom in another molecule under exactly the right conditions, knocks electrons out of it and produces an important chemical change in just that one molecule. What an ideal set-up, when chance breaks right, for causing a single gene mutation!

Actually, sexually mature fruit flies were subjected to radiation of different intensities and for different times and a dose determined empirically which would induce the maximal number of mutations. Of all the rays that passed through the bodies, only a few produced any change; of these, only a few acted on germ cells; and of these, but a small number on the genes within them; yet the natural rate of mutation was accelerated several hundred times.

Can it be that the spontaneous mutations in nature are similarly due to the random bombardment of organisms by radiations of short wave length, radiations, like cosmic rays, which reach the earth's surface from the upper atmosphere or beyond? There is at least this much evidence to support such a notion; when a well-studied population of fruit flies was bred for some generations in a deep cave, and so partly protected from these sidereal shafts, the incidence of "spontaneous" mutations was distinctly lowered. On the other hand, the amount of radiation ordinarily about us seems far from adequate to account for the normal rate of mutations.

Genes at Work

So much, then, for the genes themselves, specific individual molecules, almost surely protein, most likely enzymes, and autocatalytic to the extent that each one reduplicates itself in the course of cell division. The loci of these molecules on chromosomes within the cell is known with considerable accuracy; it is not an unreasonable expectation that, with ever more refined methods, their particular chemical structures will some day be unraveled with equal precision. Two very important problems remain. The mechanism of reduplication, the manner in which one particular complex molecule molds the surrounding raw material into its own likeness, remains at present the same mystery already seen under specific synthesis in general; even a greater one, for these molecules reproduce only

once in each cell generation. The other problem is that of the mechanism by which these catalytic molecules bring about the ultimate structures and organization of the developed organism, i. e., of how genes act. In this case the general processes are clear enough but the details are almost completely lacking.

Development, we have seen, consists of a continued sequence of individual reactions, chemical and structural. Specific substances are synthesized and properly organized in cells; totipotent cells subdivide, differentiate, and migrate to form their appropriate structures of particular composition. Each new step is based upon the preceding one; and what will happen at any temporal cross section of the developmental process seems to be determined by the past history and the current situation, by the reacting system and the stimuli playing upon it. Having responded, the whole moves on to a new state of being, which will react again in the next step according to its now determined character and the new environmental situation. If the past history of the system be considered its heredity, then indeed much environment is woven into this in the course of development.

The same situation obtains at the behavior level. What one does at any moment depends on what he is and the situation to which he is responding. But what he is at this moment is itself compounded from his previous experiences and his reactions to them as well as from the initial structural endowment received at birth. Or, at the strictly chemical level, the same again holds, for the final products of reactions depend on the initial reagents present but no less on the surrounding temperature, pressure, illumination and the like; and, of course, the secondary reactions depend on the products of the first ones and therefore on the reaction conditions as well as the ingredients of the initial mixture.

Nor are highly elaborate substances and forces indispensable for producing intricate results. Thompson has put it well: "If we blow into a bowl of soapsuds and raise a great mass of

many-hued and variously shaped bubbles, if we explode a
rocket and watch the regular and beautiful configuration of its
falling streamers, if we consider the wonders of a limestone
cavern which a filtering stream has filled with stalactites, we
soon perceive that in all these cases we have begun with an
initial system of slight complexity, whose structure in no way
foreshadowed the result, and whose comparatively simple in-
trinsic forces only play their part by complex interaction with
the equally simple forces of the surrounding medium."

So for the genes and heredity. The elegant array of catalytic
molecules present in a fertilized egg can produce no individual
without a continuous supply of a fairly extensive range of sub-
strate molecules, of foodstuffs, vitamins, and the like. Certain
substrates, under the influences of appropriate genes, are
changed into useful substances for elaboration into new struc-
tures. New genes may then act upon these new materials, or
the old genes continue to act with different effects under the
modified conditions. At all times the rate and even the direction
of change is influenced not only by gene and food, by catalyst
and substrate, but also by the conditions of reaction—tempera-
ture, acidity, concentration of a variety of salt ions, of oxygen,
and the like.

Such a picture makes it clear why single genes are so rarely
related to single body characters in a unique relationship;
rather all tend to cooperate to a greater or lesser extent in pro-
ducing the total organism. Those genes which govern early
reactions, or those of particularly great quantitative or qualita-
tive importance, will obviously have the widest influence on a
large number of traits and be the most indispensable ones. A
gene causing pigment to form in skin cells will have little im-
portance until the skin cells are present and awaiting its action
in their further development; whereas a gene responsible for
the initial differentiation of ectodermal cells, those in the outer
layer, will obviously be necessary to enable the embryo to
develop much past the blastula stage.

The appearance of organizers in embryonic development, of proteins and other specific chemicals in cell growth and multiplication, the generation of antibodies and other natural or acquired immune substances, the production of enzymes within the cells and of hormones secreted by them and, far from least, the regular grinding out of gene duplicates—all these are related steps, occurring simultaneously or sequentially in complex but determined patterns, which produce the fully functioning organism from the potent but unexpressed original cell. Genes are responsible for the early laying down of the specific cells of the thyroid—thyroid absence and cretinism is an inherited defect—the thyroid hormone in turn cooperates in the further action of the original gene to form the oxidizing enzymes of cells and so helps control their rate of growth and metabolism. Such a story can be multiplied by hundreds of analogous examples even in our present state of knowledge, but it is a matter of the distant future to discover the innumerable strands of this tapestry and how they are individually woven together to produce the ever-unfolding ever-majestic picture of living organisms.
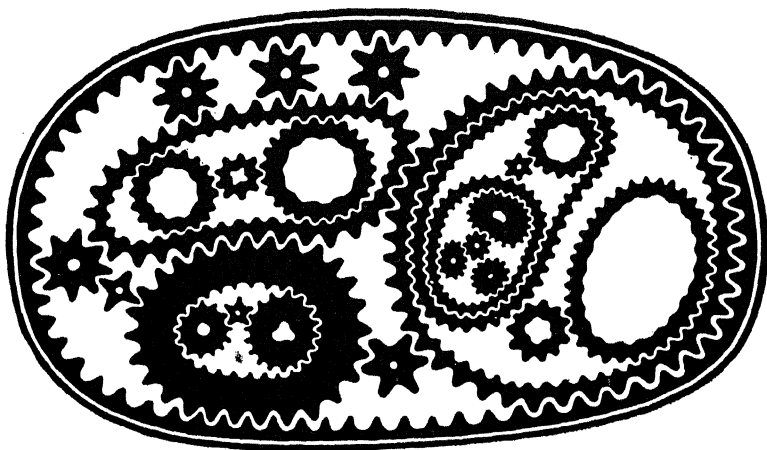
Chapter Fourteen: Organism— Epilogue

The living things we know are limited to a thin film around the earth's surface. A few yards into the ground in burrows or above it in trees and a few miles into ocean depths or airy heights includes them all. Yet in this narrow layer, between the vast depths of the earth and the vaster expanses of the atmosphere and of space beyond, teems an unbelievable variety of plants and animals and untold numbers of individuals. Over a million kinds of organisms have been discovered and classified, an unabridged dictionary of just the names of species. In every conceivable niche and cranny—in air, earth, and water; in wet and dry; hot and cold; light and dark; upon, inside of, and even within the cells and bodies of other forms— exist the host of organisms we know. Surely the casual observer or trained naturalist receives an impression of heterogeneity and striking dissimilarities rather than of any harmony in the pattern. Variation is the key word, with a profusion of plants and animals, of parts and processes, from pond scum to plum trees, from protozoa to polar bears, with metabolism and metamerism and mutation flitting across the screen of biological existence.

Yet underlying the variegated designs in the tapestry of life are the same threads as warp and woof, just as the countless substances of the non-living world are merely particular combinations and mixtures of the same few elements. Biology deals with living organisms; and if it be entitled to any dignity as a segregate of knowledge, these words at least must define the general characters of a class. Certain basic attributes and activities are present in all systems that can be called alive. Certain problems face them and have been solved. The detailed solutions are as numerous as the kinds of individuals, but a mouse and a mosquito, a marigold and a man, are still all doing essentially the same things in essentially the same way.

Life's Earmarks

All living things are semi-closed systems, partially differentiated from, partially continuous with, the environment that extends about them in time and space. These systems are organized units, organisms, and we must shortly examine still further the meaning of organization. In contrast to most other organized systems (but not all, for in a primitive way a candle flame qualifies under all the following criteria), living organisms manifest three great characteristics: they are in dynamic equilibrium, they perform specific synthesis, and they manifest adaptive amplification.

Like a whirlpool (or the candle flame), and in contrast to a stagnant pool, living entities maintain their state of being essentially constant, that is in equilibrium, only so long as they continue to divert a steady stream of matter and energy through themselves. When the flow stops the system disintegrates. To be sure, the equilibrium is not perfect and over longer spans the individual grows and ages and the stock evolves; but these are only minor drifts in the moment-to-moment rush of continued change which yet leads to constancy. The great group of chemical activities of cells and larger organisms are aspects

of this dynamic equilibrium—breathing in oxygen, digesting and absorbing the foods for it to burn, excreting the waste products and, the keystone of the arch, the actual transmutation of substances and capturing of energy during metabolism in the body cells—and are the answers to the problem of dynamic equilibrium.

Specific synthesis, a directed drift of the equilibrium, a type of autocatalysis, a form of explosion which, once started, spreads and increases by the act of exploding, comprises the phenomena of growth and reproduction, of regeneration and repair.

Adaptive amplification is the active regulated effort of the system to maintain its equilibrium or return to it when disturbed. The gyroscope that stabilizes a ship or a plane and the electrical regulator of a thermostat are set into action by small energy changes in their surroundings and, by tapping larger energy reserves of their own, combat the change and preserve the initial state. They are man-made machines which show this property. The irritability and behavior of the individual plant or animal and the slower adaptive evolution of the race, are the equivalent manifestations in the living world.

Now what of the many solutions to these basic problems? How is the requirement of feeding the machine, of maintaining its dynamic equilibrium, met by the variety of living forms? Consider, for example, the supply of water. The protoplasm of all cells in all organisms is composed overwhelmingly of a dilute salt solution, about the same one in all cases, and its water content is kept extraordinarily constant; yet the cell or individual cannot insulate its surface so as to retain safely its initial inheritance of water. Some is accidentally lost through the best waxy coat, some is necessarily lost as a solvent in which to carry away the molecules of waste products, some is continuously formed by metabolism itself. Water can and must enter and leave the cell, but the balance must be kept accurate. So far the problem is common to all; beyond it is strikingly different for many.

Paramecium lives in pond water containing almost no salt. Water steadily osmoses in to dilute its protoplasm and must be eliminated while the precious salts of its tiny body are retained. Is it surprising that a special organelle exists, the contractile vacuole, which interminably pumps out of the animate boat a "urine" which is practically pure water; that the vacuole empties itself less frequently when the animal is in a salt solution, from which the osmotic entry of water is diminished, most frequently when, in pure water, these molecules rush in at a maximal rate? The contractile vacuole can discharge a mass of water equal to the total volume of the paramecium within a few minutes.

Or consider a fresh-water crab, facing the same problem of being diluted until it bursts, as compared with a marine relative living in water with a much higher salt concentration than that of its own protoplasm. The latter is subjected to the same osmotic forces but working now in the reverse direction, and it must battle to maintain its fluid while pumping out the extra salts which diffuse into it. It will hardly surprise us to find, as we do, that the one crab excretes a very dilute urine like paramecium, the other an extremely concentrated one, with a salt content even larger than that of sea water. There are, in fact, some species of this crustacean which wander up the mouths of rivers and are sometimes in the outward flow of fresh water, at others in the inflowing tide of salt or brackish water. They can live equally well in both situations, excreting the urine appropriate for each.

If we look further, to land-dwelling forms like a man or a maple tree, the problem is still different. For these organisms, incidentally, water has still a new use as a thermal regulator; for the sun beating directly down upon the plant or animal would soon cook it except as the continuous evaporation of water from its surface, as from a Mexican ojolla jar, serves to dissipate the heat. A continuous loss of water from terrestrial organisms is therefore not only inevitable but actually necessary, and the resultant demand for this liquid is no longer met

automatically when the surrounding medium is air. The tree has solved this problem by extending ramified and individually tufted roots to tap the ground-water supply and by developing pores in its leaves to help control water loss. When the plant is losing fluid too rapidly into very dry air, these stomata shut down even though this temporarily interferes with temperature control and the entrance of carbon dioxide for photosynthesis.

Man and his congeners are not attached to a fixed source from which water is continuously taken in; some active behavior is needed to control its intake. The rate of water loss depends on metabolic activity and breathing, on food intake and excretion, and on the important requirements of body cooling; so that any fixed rate of entry would serve poorly in striking a balance. Some signal to the animal is necessary which informs it, without the aid of reason, when water is needed and when enough has been taken. The sensation of thirst is evolution's answer to this problem. Sensory messages are set up by the actual dehydration of cells in the lining of the mouth and throat and lead an animal, even when it lacks its "thinking" brain, to swallow water.

Superficially, it would seem hard to imagine more different phenomena than: the eager lapping of water by a thirsty dog, the determined closing of leaf pores, or the incessant pumping away of paramecium's contractile vacuole. But underlying these differences in behavior are the same inexorable laws of osmosis, diffusion, and evaporation, and the identical problem of maintaining the water of protoplasm at its normal level.

Or survey the problem of food. The amoeba flings itself upon a morsel, phagocytizes, and eventually digests it, while it still remains in a vacuole of water engulfed at the same time. Paramecium, more efficiently, sweeps food particles into its gullet but then their further history is the same. The crab captures its prey in its claws, chews the food with its peculiar mouth parts, and swallows the mass. It is digested in an organ called a liver, which in fact is little like the liver or the gut of

man, but which is rather a spongy secreting tissue into and out of which the watery food ebbs and flows. Man, more elegantly, has come to use a fork rather than a claw, but also chews and swallows his food. Then, in anatomically differentiated parts of his digestive system, it is squirted first with acid enzymes and then with alkaline ones to complete its chemical breakdown. And the tree has no digestive apparatus at all!

Can we still say that these different organisms are solving the same problem and in the same fundamental manner? Yes indeed. The problem is a very simple one; that of getting food molecules into the cell protoplasm. But protoplasm is surrounded by semi-permeable membranes through which colloidal molecules or larger particles can not pass. The food of all dependent organisms, plant or animal, is composed of the complex fats, proteins, and starches which constituted the bodies of other living things. These tissue masses must be comminuted, these giant molecules must be chemically dismembered, to produce small and soluble molecules able to penetrate the membrane and enter directly into the cell protoplasm. In every case, therefore, enzymes must pass out of the cell and digest the food while it is still extracellular. Surely it is but a minor difference that amoeba forms a closed pocket within itself into which digestive enzymes are secreted and from which amino acids and other building blocks are absorbed into the protoplasm; while man has as a permanent part of his anatomy a similar cavity, open through mouth and anus to the outside world and demarcated by its cellular walls from the true interior of his body, into which similar digestive enzymes are secreted and from which the same food fragments are absorbed.

Or look at the minute bacteria which, while giving no visible sign of taking food, yet slowly liquefy and remove the surrounding pabulum. The story is no different; now enzymes are secreted to the outside of the tiny body and the food there is digested and taken in without movement. But whether the food is in a cavity located within the organism but not part of it or

is lying free in the surrounding medium, the mass is digested external to the protoplasm by enzymes secreted from it; and only the soluble molecules so produced finally diffuse into the cells. Many symptoms appearing in a man suffering an infection by disease-producing bacteria are due to the action of these secreted extracellular digestive enzymes.

It is now obvious enough why the tree has no digestive apparatus—it has not the slightest use for one. It makes its own food by photosynthesis from water, carbon dioxide, and some simple salts, all small molecules already freely soluble in water and able to diffuse across the membranes of its cells—there is no problem of digestion to solve. But it might be said that, none the less, this is truly a fundamental difference between plants and animals; only the latter were clever enough to develop digestive enzymes and so live upon their less astute fellows. Oh, no; plants can do the same. The many insect-eating plants alike trap their prey by some mechanical device, are faced with the problem of digesting complex food molecules, and solve it in the familiar fashion. Into the bowl of the pitcher plant, for example, is exuded a strong digestive juice which decomposes the captured insect. The resulting nutritious fluid is then reabsorbed. The molds, smuts, and other kinds of fungi similarly move forward in their host plant or animal behind a barrage of chemical weapons which destroy and tear down the tissues ahead of them. So again in the matter of food, and despite its polyphonic variations, the same theme, of enzymic breakdown and diffusion of the small molecules produced, recurs throughout the living world.

But surely in metabolism itself there are irreconcilable differences between plants and animals. True, all organisms carry on respiration and burn foodstuffs for energy, but only the chlorophyll-rich independent plants can perform the exactly reverse process of building up foods with the aid of sunshine. Yet even here the relationships are very close; for the chlorophyll which controls sugar production and the universal respi-

ratory enzyme which is necessary to its oxidation, even the hemoglobin which brings to vertebrate tissues the rich supply of oxygen they require, are practically the same stuff. To be sure, the proteins in these molecules differ, but so they do from one species of animals to another; the crucial chemical group— a large, intricate edifice built of carbon, nitrogen, oxygen, and hydrogen in four interlocking rings, a tetrapyrrol or porphyrin —is the same in all. There are only trivial differences in the small tags of atoms attached to this great nucleus and in the metal atom set gem-like in its center. Chlorophyll contains magnesium and hemoglobin, iron, but the one metal can readily be substituted for the other.

And a vast number of the catalytic substances of plant and animal cells are identical. The ascorbic acid which prevents scurvy was first found in cabbage leaves; later crystals of the substance were obtained from adrenal glands. Clearly, it is not present in the cabbage to make this a more adequate food for animals, but to serve in the metabolic processes of the plant as it does in ours. Perhaps even more clear is the situation for yeast and muscle. When some of the enzymes and their aides essential to fermentation by muscle are destroyed by extracting the cells in various ways, these missing units can be replaced by adding other muscle extracts which contain them and fermentation be restored. The activity of the partially disrupted muscle system can, moreover, be reinitiated by similar extracts made from yeast; and the activity of injured yeast be restored by muscle juices. This can only mean that some of the yeast catalysts, which ordinarily break sugar to alcohol, can fit into the enzyme system of muscle and help to produce its usual lactic acid. There are interchangeable parts in the two machines; some of the wheels from the yeast mechanism are identical with some in that of muscle, else the hybrid engine could not run.

After all, metabolism cannot be so utterly different in various organisms, for the widest variety of them depend upon glucose

as their primary fuel. It is a minor difference only that muscle breaks this sugar into two molecules of lactic acid while yeast carries the process a little further and, in effect, breaks each lactic acid molecule into one of alcohol and one of carbon dioxide. The problem of metabolism is to release for cell work the free energy stored in the molecules of food substances. This requires the chemical activation of these molecules and their ultimate combination with oxygen, under the influence of cell enzymes and carriers.

To be sure, many other problems arise and the processes of metabolism have been improved in various organisms. Warm-blooded animals, for example, have evolved their complex thermostatic regulators primarily to maintain the metabolic rate of their cells more nearly constant and to make it independent of the widely fluctuating climate in which they live. Further, the larger multicellular organisms, especially the higher animals, have had to develop circulatory systems able to bring food and oxygen to the door of the individual body cell far more rapidly than could the slow process of diffusion from the body surface. And to obtain sufficiently rapid entry of oxygen into the circulating fluid another series of problems had to be met and respiratory organs were developed. But here again, whether through skin or gills or lungs or ramifying air tubes, the same process occurs in the same way. The circulating fluid passes through many fine capillaries, in which it is separated from the outside oxygen by the thinnest possible membrane with the largest possible surface. The oxygen-containing medium is also continuously renewed, and this gas then simply diffuses across the membrane from the fresh air or water, as the case may be, into the rushing blood.

No, there are no basic differences between living organisms in their maintenance of dynamic equilibrium. The same sort of food runs the same chemical gauntlet in the same structural machine. The energy so obtained is routed along like paths to similar uses. The living machine is fed in the same manner, however varied the body that houses it.

Does the building of the machine, specific synthesis, show the same basic homogeneity throughout the living world? An increase in the amount of specific materials in organisms is required for an increase in size of the individual, growth; for an increase in the number of individuals, reproduction (surely it is not primary whether reproduction goes through the machinery of gametes or spores or by vegetative growth and division of other body parts); and even for an increase in complexity, as occurs during development. (When a fertilized egg becomes a blastula the total mass of protoplasm may have actually decreased, yet many small cells exist in place of a single large one. This advancing differentiation demands, of course, the synthesis of more materials, such as those which form nuclei, the volume of which is much increased, or those which compose cell membranes, also multiplied.) To form these additional quantities of the constituents of protoplasm, enzymes are needed; to make and increase these catalysts other enzymes are required; until finally the responsibility is centered on a larger or smaller group of autocatalytic molecules.

Their activity we have seen illustrated over and over again. The growth curves of a colony of bacteria, of a population of flies or humans, and of the weight of a multicellular individual (a cell colony) are alike and measure the rate of increase of their active enzymes. The hypertrophy of an organ with function, the modification of behavior by experience or learning, perhaps even the slow trend of evolutionary advance, are more or less closely similar phenomena involving autocatalytic change. The virus protein, whose presence causes plant cells to build more of it than of their own proper proteins, illustrates specific synthesis in its purest form. The antibodies, which overflow into the intercellular fluids from the phagocytic cells, are likewise autocatalytically generated enzyme molecules of the highest specificity. Only that kind of protein which has earlier been taken up and digested by these cells is subsequently attacked by the antibodies liberated from the cells as a consequence of this digestion. The intracellular enzymes, hav-

ing operated upon a given protein, become increased in amount and so the antibodies which destroy that particular one. Most vital of all specific syntheses, of course, are those involving the genes themselves; for the reduplication of genes in the course of mitosis is the basic event of cell multiplication. It is absolutely essential that each daughter unit obtain one full set of the gene enzymes, and the elaborate chromosome machinery is merely an especially foolproof means of dealing them out.

But in their curves of growth and development, in their immunological defense against disease, in their chromosome machinery, plants and animals, flatworms and giraffes, behave fundamentally alike. Yes, the living machine builds itself according to the same chemical blueprints whenever it builds.

Behavior

Building and feeding the living machine are always managed with the same physico-chemical tricks. Does this hold for running it, for adaptive amplification? The behavior of an entire organism, more than any other of its activities, depends upon its organization, from the most detailed to the most inclusive levels. This is the mechanism for utilizing its energy toward useful ends by adaptive behavior. "Useful" and "adaptive" imply such action as tends to continue the equilibrium state intact, to maintain organization in the face of disrupting forces, to preserve the delicate balance of life against disturbing environmental change.

An imposed change from the organism's surroundings must evoke, at its inception, an adequate counterchange in the organism. A small amount of applied energy, a weak stimulus, must elicit the prompt liberation of a larger amount of energy, an effective response, correctly directed as adaptive behavior. Some amplification must occur along the way, often a great deal, and the stimulus so serves as a trigger to release previously stored and readily mobilized reserves.

The picture is not unlike that of a radio receiver which applies a reserve of energy, from battery or wall plug, to the amplification of a feeble incoming electromagnetic signal and produces an audible sound. It does more than magnify for, also like an organism and through its special organization, it directs the energy flow and it stabilizes itself. A good instrument differs from a poor one in that: it is more sensitive and responds to weaker signals (has a lower threshold); it is more selective and picks out one frequency from more closely similar ones (has a finer discrimination); it is of wider range and picks up longer and shorter wave lengths (has more senses); and it gives a better performance with more balance and richer overtones (has a more coordinated behavior). Further, these differences are only in part due to finer materials—condensers, tubes, and the like—in the superior set. Far more important is the complexity and elegance of their combination into an organized whole, the circuit design.

For organisms, also, improved performance is only in part predicated upon improved materials. Vertebrate striated muscle cells are certainly more efficient instruments for movement than are the lumbering cells which guard the stomata of leaves or even the sluggish smooth muscle cells of the jellyfish; and our medullated nerve fibres carry messages with a dispatch and precision beyond the capacities of the primitive naked nerves of a crab, and of an entirely different order from those which creep along the hardly differentiated protoplasmic strands of the sensitive plant. Yet the real basis of the difference in behavior of a man and a mouse, of a mussel and a mushroom, is not in the materials (after all only more or less elaborated protoplasm) of which the organisms are built, but rather in the intricacy of organization of these materials.

If there is any sense in which we may properly speak of "higher" and "lower" in the living world, any sense to the assertion that man represents the peak of animate evolution, it is in terms of the perfection of the individual's organization and

the attendant responsiveness to the surrounding world. Man is more sensitive to and more discriminating of more kinds of stimuli than is any other plant or animal, and he can respond to them with a more complex and integrated behavior. On such criteria he may indeed claim to be the king of beasts and the lord of creation.

Fundamentally, the behavior of the living machine, adaptive amplification, is alike in all organisms; but in this third universal attribute of living beings, far more than in the other two, maintenance and increase, the range is so extreme that some qualitative differences seem to creep in. The impulse which races along a nerve or through the labyrinthine pathways of a brain is merely an improved model of the electrochemical change which crawls along the surface of any mass of protoplasm. Yet the advent of a nervous system and its steady development have conferred seemingly new qualities upon its possessors.

It is a far cry from the tropistic bending of plants and the sol-gel performance of an excited amoeba, through the convulsive generalized collapse of a disturbed sea anemone, to the perfectly coordinated running movements of a stimulated dog. The more elaborate behavior parallels a more elaborate nervous system; from none, through a scattered net of nerve cells, to a magnificently organized central nervous system with its myriad synaptic connections set and its many coordinated reflexes predetermined. For predetermined and automatic they are—a dog with its entire cerebrum removed can run with as great agility as can a normal animal, since the whole sequence of muscle contractions and relaxations depends upon successive unthought reflexes each initiated by its predecessor.

But it is perhaps an even greater jump from the dog without a cerebrum to one with it, or to man, than from the amoeba to the decerebrate mammal. For, with the cerebral cortex, there clearly emerge qualities of behavior which were only dimly foreshadowed in more primitive animals—the qualities of modi-

fiability, of learning, of choice, and ultimately those of analyzed awareness, of abstract and symbolic reasoning, and of pre-science. Somehow, the few ounces of additional nerve cells built into the gray shell of the human brain have pointed up the potentialities of voluntary and rational behavior to an unprecedented level.

Pointed up, but not created. There is no more a sharp demar-cation between thoughtful and automatic acts, between con-scious and insensate states, than there is between living and inanimate systems. When complexity at one level of organiza-tion becomes sufficient, the new permutations are so great that there seems to be a qualitative discontinuity, a new emergence. But we must consider organism, as such, more fully before skirting the morass of free will and purposeful evolution.

The Whole

Organism is the machine itself. An organism, whether or not a living one, is an integrated system composed of interre-lated units. The individual water molecules darting about in air are units and the many billions of them form a collection or swarm or mob but, being in no essential relation to one another, they do not constitute an organism. The same molecules of water vapor crystallized into a snowflake are interrelated and organized—the position of one is related to those of its fellows and all are fixed by intermolecular attractions—the snowflake is an organism. Similarly, deer hunters separately roaming a forest remain an aggregation of single units, essentially inde-pendent despite an occasional accidental shooting. But the same men and guns, when marshaled into formations and per-forming directed maneuvers, become a small army, a new organism with properties quite different from those of the scattered hunters.

Obviously, at any one level, units may be knit loosely or closely into organisms of low or high degree. Molecules in a

wind constitute more of an organism than those in still air, for they share a common directional component of motion. Those in a liquid, still more in a solid, are more fully organized, their individual behavior being more dependent upon the presence of their fellow molecules. Still more elaborate organisms are the colloidal solutions, with surfaces of micelles and membranes adding new attributes to the system. These, then, represent a progressive series of more perfect organisms at the molecular level of organization.

But organisms may differ in level as well as in degree of integration, and one can be built upon another as are the layers of an onion. Electrons, neutrons, and protons become organized into atoms, which are, therefore, organisms. These atomic individuals are, however, new units and in turn form systems of a higher order, molecular organisms. These, as still new units, are integrated to form the units of protoplasm and so of cells. And cells are the units from which are built, in ever-ascending levels of organization, the tissues, organs, systems, and at last the complete multicellular individual. Further, individuals often live in interrelation in definitive groups—the colony, family, herd, clan, society—each of which thus becomes in varying degree an organism. (If the more general organized systems be called orgs, then living ones would be animorgs. Organism would then retain the meaning of a single living individual, and epiorganism be used for societies of such individuals.)

At the cellular level, amoeba is a more advanced organism than a bacterium, which lacks even a nucleus; and paramecium, with its fixed organelles and coordinated cilia, is still more advanced. The criterion of advance is, of course, the degree of interdependence of the parts of the organism. This implies differentiation of the units and their reintegration. The more unique each unit is, the more specialized the role it plays, the greater must be the dependence of each on the others and the more intimate the coordination of the many into a single func-

tioning whole. On the same bases, the sponge is a very primitive organism at the multicellular level, though its individual cell units are fully as advanced at the unicellular level as is paramecium. The sponge can be squeezed through fine mesh cloth, yet the separated cells will reclump, or regenerate any missing comrades, little the worse for their experience. Clearly there is no great interdependence from cell to cell—imagine a similar experiment with man!

Higher organisms are built of more highly differentiated units which are more perfectly integrated into the new whole. Evolution has steadily given rise to more integrated systems at any one level and to new systems at more complex levels of integration. At the cellular stage, the simple alga with almost homogeneous protoplasm has been supplemented with other organisms, as paramecium, possessing many highly differentiated organelles. But a second level has also been introduced, starting with the little differentiated cells of sponges or seaweeds and progressing, still at this multicellular level, to the specialized interdependent cell group which constitutes a mammal. The next level also appeared early, for the society, the epiorganism, can be recognized perhaps in protozoan colonies, is striking in the colonial coelenterates (a coral reef is such an epiorganism), and has reached one type of perfection in such social insects as ants or termites.

Multicellular organisms, those usually meant by the unqualified word "organism," advance in efficiency and in unity as the mechanisms integrating their many cells improve. Plants and sponges depend upon the sluggish diffusion of chemicals or the laggard propagation of excitation by unspecialized protoplasm for communication from cell to cell, and it is very inefficient. In flatworms, diffusion is aided by some stirring and churning of fluids, produced accidentally by muscle movements; protoplasmic transmission is junked in favor of nerve pathways of a primitive type; and metabolic gradients are important in the overall stabilization. In man, a directed and

efficient circulation carries substances around his entire body in well under a minute; and a whole battery of organs has evolved, the glands of internal secretion, which utilizes this pumping system to distribute special substances which control and integrate his varied body parts. Further, an elaborate visceral nervous system speeds messages about within his body and a great central nervous system, capped by his priceless cerebral cortex, controls his behavior as a total individual.

In the epiorganism, likewise, the integrating mechanisms evolve and more advanced societies appear. The stages of chemical coordination, involving transportation, can be likened to mail delivery by stagecoach, railroad, and airplane; those of neural coordination, involving transmission, to shouts, smoke signals, and the telephone. Clearly there are social gradients which parallel the metabolic ones; and newspapers, motion pictures, and radio are progressively increasing the extent and intensity of such mass control. The societies with the more effective integrating mechanisms are the more closely knit and spatially fixed—Arabia, with its scattered and self-sufficient nomad groups, is rather spongelike; highly mechanized England, nobility gradient and all, has perhaps reached the flatworm stage. But in all cases the differentiation of individual units—into merchants, lawyers, beggarmen, and thieves—seems to have outrun the forces of coordination; and most current species of human epiorganisms seem to be suffering severe growing-pains.

The crux of "organism" is in the interplay and control existing among the parts and between parts and whole. Our common thinking about control or determination has a time dimension. Attention is usually directed to events which occur in temporal sequence, often with regular repetition. When a given event is most easily interpreted in relation to a regularly occurring antecedent one, the latter is said to be the cause of that given one. "A solid particle in the windpipe causes a cough." When a particular event is best understood in terms of a subse-

quent one, the latter is called the purpose of that particular one. "He coughed on *purpose* to attract her attention." But an organism is a state of being, as well as of becoming; the mechanisms for integrating its unit parts into a single whole must be in maintained existence and some at least must be independent of time, like gravity or quantitative gradients. These steady states or fields may themselves be dynamic equilibria, dependent on whirling electrons or burning glucose, but while they endure time's arrow has no point.

A cell in an organism is different from one alone and is so by virtue of its being part of a whole. An amoeboid leucocyte moves into an infected tissue and dies, a free-living amoeba moves from like conditions and lives. The reciprocal control between parts and whole is peculiarly difficult to lay hold of but is none the less essential. This is well illustrated in the problem of aging; cells become old when in the tissue fluids of an old body, but the body, and fluid, is old because it is formed by old cells. The cycle of determination is partly temporal, partly not.

Similarly for the number of nerve fibres growing into a limb; for the parallel orientation of micelles and fibres in muscle; for the arrangement of bone trabeculae along curves of stress; for the hypertrophy of one bit of adrenal gland when the remainder has been lost; for case after case in which structure and function of colloids, cells, and tissues are molded into conformity with the requirements of the whole organism. The story is apposite of the hod-carriers questioned about their work. One replied, "I am carrying bricks"; the second, "I am making a living"; the third, "I am helping to build a cathedral." Cells in a multicellular individual are helping to build and maintain the whole magnificent structure, and this has an integrity which transcends and is partly independent of any particular cell or other subdivision.

So for the problem of volition and free will. The argument from electron indeterminism, by amplification through several

levels to individual behavior, gives only indeterminism of action —which is chaos rather than choice or purpose. Volition implies some sort of control of its units by the organism; the power to compel ion movements in nerve cells and the discharge of nerve impulses to appropriate muscles. What such a control can be is at present unimagined. Perhaps our subjective conviction of willing our acts is a complete illusion, certainly it is an almost complete one or else great fields of physiological and psychological evidence are woefully in error. But perhaps some volition is real and represents a universal ability of any total organism to control its constituent units, a control present in atoms and protozoa and only given special emphasis and point by the amazing nervous system and its crowning cerebral cortex.

Now a final word. The second law of thermodynamics operates universally to disorganize all exisiting systems, to make the universe more random and mixed up. Yet living systems continuously prevail against this trend and increase their orderliness or organization. In the course of individual development, from relatively homogeneous egg to highly differentiated adult; in the course of recovery from action, with rebuilding of membranes and restoring of potential energy; in the whole mighty sweep of evolution, from a microscopic blob of protoplasm to the magnificently elaborated organisms of our daily experience, living things show a steady and seemingly irresistible trend toward increasing order. But this organized state is not a static one; free energy is continuously being destroyed in the surroundings though increasing in the organism.

The achievement of animate beings is not recorded in some permanent monument, like the great pyramid, the huge rocks of which, once laboriously hauled to the apex, triumphantly retain their position for a short eternity. It is itself a dynamic equilibrium, the maintained hovering of the bird or airplane in air, lifting itself by continued action against the forces forever stretching to drag it down. A small breakdown in the living

machinery, a single false move in behavior, a brief remission of control, and individual or species plummets down to death and oblivion. The path of living things is strewn with its failures, the forces of disintegration are continually eroding its ranks, but the parade of the living marches on—and up.

For the epiorganism, likewise, "eternal vigilance is the price of freedom"; and, though the present forms of civilization look discouraging indeed, they are still a manifestation of that life process which has always pulled itself up by its bootstraps, by means of autocatalysis, mutation selection, and the like. Differentiation of units, division of labor, mutual cooperation, and integration into a larger whole have survival value; for as organisms have improved in such organization so have they prospered. Mutual helpfulness of individual units and sacrifice of the lesser unit for the good of the greater are real biological virtues. It is no accident that they are also social ones. Many, perhaps all, of the present primitive species of society will not make the grade and will disappear; but not before others have derived from them. The huge weight of biological experience must give us a strong hope that civilization is not doomed to destruction. Human society will survive and will improve.

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